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=> s (chimeric enzyme or variant? or mutant? or target molecule) and (antibod?
or binding molecule)

2 FILES SEARCHED...

L1 45408 (CHIMERIC ENZYME OR VARIANT? OR MUTANT? OR TARGET MOLECULE) AND
(ANTIBOD? OR BINDING MOLECULE)

=> s l1 and (active domain or inactive domain) and beta lactamase?

L2 0 L1 AND (ACTIVE DOMAIN OR INACTIVE DOMAIN) AND BETA LACTAMASE?

=> s l1 and (active or inactive enzyme) and beta lactamase?

L3 3 L1 AND (ACTIVE OR INACTIVE ENZYME) AND BETA LACTAMASE?

=> d l3 1-3

L3 ANSWER 1 OF 3 MEDLINE
AN 1998060838 MEDLINE
DN 98060838
TI Peroxisomal targeting, import, and assembly of alcohol oxidase in Pichia
pastoris.
AU Waterham H R; Russell K A; Vries Y; Cregg J M
CS Department of Biochemistry and Molecular Biology, Oregon Graduate
Institute of Science and Technology, Portland, Oregon 97291-1000, USA.
NC DK-43698 (NIDDK)
SO JOURNAL OF CELL BIOLOGY, (1997 Dec 15) 139 (6) 1419-31.
Journal code: HMV. ISSN: 0021-9525.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
QS GENBANK-U96967; GENBANK-U96968
EM 199803
EW 19980303

L3 ANSWER 2 OF 3 CAPLUS COPYRIGHT 1999 ACS

AN 1998:388600 CAPLUS
 DN 129:38110
 TI **Chimeric enzyme** molecules having a regulatable activity for use in assays
 IN Legendre, Daniel; Soumillion, Patrice; Fastrez, Jacques
 PA Universite Catholique De Louvain, Belg.
 SO PCT Int. Appl., 62 pp.
 CODEN: PIXXD2
 DT Patent
 LA English
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9823731	A2	19980604	WO 97-IB1643	19971126
	WO 9823731	A3	19981112		
	W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
	RW:	GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
	AU 9855719	A1	19980622	AU 98-55719	19971126
PRAI	US 96-757425		19961127		
	WO 97-IB1643		19971126		

L3 ANSWER 3 OF 3 BIOSIS COPYRIGHT 1999 BIOSIS
 AN 1989:309739 BIOSIS
 DN BA88:23469
 TI INVESTIGATION OF THE ABILITY OF NEWER BETA LACTAM ANTIBIOTICS TO SELECT RESISTANT **MUTANTS** FROM SERRATIA-MARCESCENS AFTER MUTAGENESIS WITH NITROSOGUANIDINE.
 AU VUYE A; BAERT E; KERSTERS K; DECLERCK D; VANHAECKE E; PIJCK J
 CS STATE UNIV. GENT., LAB. PHARMACEUTICAL MICROBIOL., HARELBEKESTR. 72, B-9000 GENT.
 SO ARZNEIM-FORSCH, (1989) 39 (4), 424-427.
 CODEN: ARZNAD. ISSN: 0004-4172.
 FS BA; OLD
 LA English

=> d 13 1-3 ab

L3 ANSWER 1 OF 3 MEDLINE
 AB Alcohol oxidase (AOX), the first enzyme in the yeast methanol utilization pathway is a homooctameric peroxisomal matrix protein. In peroxisome biogenesis-defective (pex) **mutants** of the yeast *Pichia pastoris*, AOX fails to assemble into **active** octamers and instead forms inactive cytoplasmic aggregates. The apparent inability of AOX to assemble in the cytoplasm contrasts with other peroxisomal proteins that are able to oligomerize before import. To further investigate the import of AOX, we first identified its peroxisomal targeting signal (PTS). We found that sequences essential for targeting AOX are primarily located within the four COOH-terminal amino acids of the protein leucine-alanine-arginine-phenylalanine COOH (LARF). To examine whether AOX can oligomerize before import, we coexpressed AOX without its PTS along with wild-type AOX and

determined whether the **mutant** AOX could be coimported into peroxisomes. To identify the **mutant** form of AOX, the COOH-terminal LARF sequence of the protein was replaced with a hemagglutinin epitope tag (AOX-HA). Coexpression of AOX-HA with wild-type AOX (AOX-WT) did not result in an increase in the proportion of AOX-HA present in octameric **active** AOX, suggesting that newly synthesized AOX-HA cannot oligomerize with AOX-WT in the cytoplasm. Thus, AOX cannot initiate oligomerization in the cytoplasm, but must first be targeted to the organelle before assembly begins.

L3 ANSWER 2 OF 3 CAPLUS COPYRIGHT 1999 ACS

AB The present invention relates to a chimeric target mol. having an activity

which can be regulated or modulated by a binding mol. The invention also relates to methods of using the chimeric target mol. to detect the presence and/or amt. of a desired analyte in a sample. The analyte is a binding mol., or a competitor of a binding mol., which binding mol., upon binding to the target mol., alters the activity of the target mol. in a detectable way. In one aspect of the invention, a binding mol. binds to the chimeric mol., inactivating it. An analyte in a test sample competes and/or displaces the binding mol. from the chimera, reactivating it. The reappearance of activity in the presence of the analyte indicates its existence in the test sample, existence and amt. Another aspect of the invention relates to a binding mol. which regulates a chimeric target

mol.

and methods of producing it. Thus, random peptide sequences are inserted in loop on the rim of the **active** site (residues 103-105) or the .alpha.11 helix (residues 271-272) of R-Tem **.beta.-lactamase**. A large library of chimeric **.beta.-lactamases** is then selected for binding by monoclonal **antibodies** psa10 and psa66. One of the best regulated clones (p66Rb316) is inhibited by 68 or 75% on PADAC or Centa substrates, resp., and has the wild-type residues E104-Y105 replaced by T104G105, wild-type T271 replaced by DGSRQ, and R275 mutated to Q275. These sequences in the prostate-specific antigen (PSA), and consequently, the monoclonal **antibody** psa66 recognizes a mimotope in the **chimeric enzyme**. Similarly, selection with psa19 yields a clone with wild-type T271 replaced by SWPVKS and R275.fwdarw.Q275, which is also a mimotope structure. The chimeric **.beta.-lactamase** and **antibodies** can be used in a PSA-specific assay.

L3 ANSWER 3 OF 3 BIOSIS COPYRIGHT 1999 BIOSIS

AB Resistant **mutants** could easily be selected from a nitrosoguanidine-treated culture of *Serratia marcescens* with piperacillin,

cefotaxime, cefoxitin, cefotetan, latamoxef (moxalactam) and aztreonam. Imipenem on the other hand was significantly less effective in **mutant** selection. Resistant clones broadly fell into two distinct classes. Most **mutants** did not show increased **.beta.-lactamase**; their resistance seemed to be due to changed outer membrane proteins. Other **mutants** had strongly increased cephalosporinase activity, although the derepression was only partial. Piperacillin, cefotaxime and aztreonam preferentially selected the derepressed phenotype, whereas **mutants** selected with cefoxitin, cefotetan, moxalactam and imipenem were exclusively of the non-derepressed

phenotype. There was a significant degree of cross-resistance between the **.beta.-lactam antibodies** except imipenem which was only slightly

less **active** against the membrane-altered **mutants**.

=> s chimeric and (protein or enzyme) and target and binding molecule?

1 FILES SEARCHED...

3 FILES SEARCHED...

L4 0 CHIMERIC AND (PROTEIN OR ENZYME) AND TARGET AND BINDING
MOLECULE

?

=> s (chimeric or variant) and (protein or enzyme) and target and binding
molecule?

1 FILES SEARCHED...

3 FILES SEARCHED...

L5 0 (CHIMERIC OR VARIANT) AND (PROTEIN OR ENZYME) AND TARGET AND
BINDING MOLECULE?

=> s (chimeric or variant) and (protein or enzyme) and target? and binding?

1 FILES SEARCHED...

3 FILES SEARCHED...

L6 2156 (CHIMERIC OR VARIANT) AND (PROTEIN OR ENZYME) AND TARGET? AND
BINDING?

=> s l6 and beta lactamase

L7 17 L6 AND BETA LACTAMASE

=> d l7 1-17 ibib ab

L7 ANSWER 1 OF 17 MEDLINE

ACCESSION NUMBER: 96272150 MEDLINE

DOCUMENT NUMBER: 96272150

TITLE: The import receptor for the peroxisomal **targeting**
signal 2 (PTS2) in *Saccharomyces cerevisiae* is encoded by
the PAS7 gene.

AUTHOR: Rehling P; Marzioch M; Niesen F; Wittke E; Veenhuis M;
Kunau W H

CORPORATE SOURCE: Institut fur Physiologische Chemie der Ruhr-Universitat
Bochum, Medizinische Fakultat, Germany.

SOURCE: EMBO JOURNAL, (1996 Jun 17) 15 (12) 2901-13.

Journal code: EMB. ISSN: 0261-4189.

PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199610

AB The import of peroxisomal matrix proteins is dependent on one of two
targeting signals, PTS1 and PTS2. We demonstrate in vivo that not
only the import of thiolase but also that of a **chimeric**
protein consisting of the thiolase PTS2 (amino acids 1-18) fused
to the bacterial **protein beta-lactamase** is
Pas7p dependent. In addition, using a combination of several independent
approaches (two-hybrid system, co-immunoprecipitation, affinity

chromatography and high copy suppression), we show that Pas7p specifically interacts with thiolase in vivo and in vitro. For this interaction, the N-terminal PTS2 of thiolase is both necessary and sufficient. The specific binding of Pas7p to thiolase does not require peroxisomes. Pas7p recognizes the PTS2 of thiolase even when this otherwise N-terminal **targeting** signal is fused to the C-terminus of other proteins, i.e. the activation domain of Gal4p or GST. These results demonstrate that Pas7p is the **targeting** signal-specific receptor of thiolase in *Saccharomyces cerevisiae* and, moreover, are consistent with the view that Pas7p is the general receptor of the PTS2. Our observation that Pas7p also interacts with the human peroxisomal thiolase suggests that in the human peroxisomal disorders characterized by an import defect for PTS2 proteins (classical rhizomelic chondrodysplasia punctata), a functional homologue of Pas7p may be impaired.

L7 ANSWER 2 OF 17 MEDLINE

ACCESSION NUMBER: 95103534 MEDLINE

DOCUMENT NUMBER: 95103534

TITLE: Development of a humanized disulfide-stabilized anti-pl85HER2 Fv-**beta-lactamase** fusion protein for activation of a cephalosporin doxorubicin prodrug.

AUTHOR: Rodrigues M L; Presta L G; Kotts C E; Wirth C; Mordenti J; Osaka G; Wong W L; Nuijens A; Blackburn B; Carter P

CORPORATE SOURCE: Department of Cell Genetics, Genetech Inc, South San Francisco, CA 94080-4990.

SOURCE: CANCER RESEARCH, (1995 Jan 1) 55 (1) 63-70. Journal code: CNF. ISSN: 0008-5472.

PUB. COUNTRY: United States Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; Cancer Journals

ENTRY MONTH: 199504

AB The humanized anti-pl85HER2 antibody, humAb4D5-8, has completed Phase II clinical trials for pl85HER2-overexpressing breast cancer. Here, this antibody is used as a building block to engineer a disulfide-linked Fv (dsFv) **beta-lactamase** fusion protein for use in antibody-dependent enzyme-mediated prodrug therapy using cephalosporin-based prodrugs. Three Fv variants were designed with an interchain disulfide bond buried at the VL/VH interface and secreted from *Escherichia coli*. One variant, dsFv3 (VL L46C VH D101C0, has similar affinity for antigen ($K_d = 0.7$ nM) as the wild-type Fv and was used to construct a fusion protein in which **beta-lactamase**, RTEM-1, is joined to the carboxy terminus of VH. The dsFv3-**beta-lactamase** fusion protein secreted from *E. coli* efficiently activates a cephalothin doxorubicin prodrug (PRODOX, $k_{cat}/K_m = 1.5 \times 10(5)$ s⁻¹ M⁻¹). PRODOX is approximately 20-fold less toxic than free doxorubicin against breast tumor cell lines SK-BR-3 and MCF7, which express pl85HER2 at elevated and normal levels, respectively. Prebinding the dsFv3-**beta-lactamase** fusion protein specifically enhances the toxicity level of PRODOX to that of doxorubicin against SK-BR-3 but not MCF7 cells. The fusion protein retains both antigen-binding plus kinetic activity in murine serum and is cleared rapidly as judged by

pharmacokinetic analysis in nude mice (initial and terminal half-lives of 0.23 and 1.27 h, respectively). Development and characterization of the dsFv3-**beta-lactamase** fusion **protein** is an important step toward **targeted** prodrug therapy of p185HER2-overexpressing tumors.

L7 ANSWER 3 OF 17 MEDLINE
ACCESSION NUMBER: 93107054 MEDLINE
DOCUMENT NUMBER: 93107054
TITLE: Metalloregulated expression of the ars operon.
AUTHOR: Wu J; Rosen B P
CORPORATE SOURCE: Department of Biochemistry, Wayne State University School of Medicine, Detroit, Michigan 48201..
CONTRACT NUMBER: AI19793 (NIAID)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Jan 5) 268 (1) 52-8.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199303

AB The plasmid-borne arsenical resistance (ars) operon encodes an arsenical-translocating ATPase and confers resistance to antimonials and arsenicals in Escherichia coli by extrusion of the toxic compounds from the cytosol. The trans-acting regulatory ArsR **protein** was shown to bind to a fragment of DNA containing the ars promoter. Hybrid formation of the ArsR **protein** with a ArsR-**beta-lactamase** **chimeric protein** suggested that the active form of the ArsR repressor is a dimer. From footprinting analysis the **binding** site was defined as a region of imperfect dyad symmetry just upstream of the -35 site. In vivo the operon was derepressed by oxyions of +III oxidation state of arsenic, antimony, and bismuth, as well as arsenate (As(V)), whereas in vitro ArsR **protein**-operator interaction was reduced by each of those compounds except arsenate, as determined by gel retardation and DNase I and hydroxyl radical footprinting experiments. This indicates that arsenate is not a true inducer and must be reduced to arsenite in vivo to induce. An operator mutant obtained by deletion of the

in vitro ArsR-protected DNA sequence exhibited constitutive ars promoter activity, demonstrating that the **binding** site is the functional **target** for the ArsR repressor in vivo.

L7 ANSWER 4 OF 17 MEDLINE
ACCESSION NUMBER: 93014215 MEDLINE
DOCUMENT NUMBER: 93014215
TITLE: Fusion proteins containing the A2 domain of cholera toxin assemble with B polypeptides of cholera toxin to form immunoreactive and functional holotoxin-like chimeras [published erratum appears in Infect Immun 1993 Mar;61(3):1168].
AUTHOR: Jobling M G; Holmes R K
CORPORATE SOURCE: Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814..
CONTRACT NUMBER: AI31940-01 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (1992 Nov) 60 (11) 4915-24.
Journal code: G07. ISSN: 0019-9567.
PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; Cancer Journals
 ENTRY MONTH: 199301

AB Cholera enterotoxin (CT) is produced by *Vibrio cholerae* and excreted into the culture medium as an extracellular **protein**. CT consists of one A polypeptide and five B polypeptides associated by noncovalent

bonds, and CT-B interacts with CT-A primarily via the A2 domain. Treatment of CT with trypsin cleaves CT-A into A1 and A2 fragments that are linked by a disulfide bond. CT-B binds to ganglioside GM1, which functions as the plasma membrane receptor for CT, and the enzymatic activity of A1 causes the toxic effects of CT on **target** cells. We constructed translational fusions that joined foreign proteins via their carboxyl termini to the A2 domain of CT-A, and we studied the interactions of the fusion proteins with CT-B. The A2 domain was necessary and sufficient to enable bacterial alkaline phosphatase (BAP), maltose-binding **protein** (MBP) or **beta-lactamase** (BLA) to associate with CT-B to form stable, immunoreactive, holotoxin-like chimeras. Each holotoxin-like chimera was able to bind to ganglioside

GM1. Holotoxin-like chimeras containing the BAP-A2 and BLA-A2 fusion proteins had BAP activity and BLA activity, respectively. We constructed BAP-A2 mutants with altered carboxyl-terminal sequences and tested their ability to assemble into holotoxin-like chimeras. Although the carboxyl-terminal QDEL sequence of the BAP-A2 fusion **protein** was not required for interaction with CT-B, most BAP-A2 mutants with altered carboxyl termini did not form holotoxin-like chimeras. When holotoxin-like chimeras containing BAP-A2, MBP-A2, or BLA-A2 were synthesized in *V. cholerae*, they were found predominantly in the periplasm. The toxin secretory apparatus of *V. cholerae* was not able, therefore, to translocate these holotoxin-like chimeras across the outer membrane.

L7 ANSWER 5 OF 17 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1998:388600 CAPLUS

DOCUMENT NUMBER: 129:38110

TITLE: **Chimeric enzyme** molecules having a regulatable activity for use in assays

INVENTOR(S): Legendre, Daniel; Soumilion, Patrice; Fastrez, Jacques

PATENT ASSIGNEE(S): Universite Catholique De Louvain, Belg.

SOURCE: PCT Int. Appl., 62 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9823731	A2	19980604	WO 97-IB1643	19971126
WO 9823731	A3	19981112		
W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR,			

AU 9855719	A1 19980622	AU 98-55719	19971126
PRIORITY APPLN. INFO.:		US 96-757425	19961127
		WO 97-181643	19971126

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L7 ANSWER 6 OF 17 CAPLUS COPYRIGHT 1999 ACS

AB The heterologous surface expression of the cholera toxin B subunit (CTB) from *Vibrio cholerae* in two staphylococcal species, *Staphylococcus xylosus*

and *Staphylococcus carnosus*, has been investigated. The gene encoding

native CTB (103 amino acids) was introduced into gene constructs encoding **chimeric** receptors designed to be translocated and anchored on the outer cell surface of the staphylococci. Since functionality of CTB is correlated with its ability to form pentamers and the capacity of the pentameric CTB to bind the GM1 ganglioside, both the surface accessibility

and the functionality of the surface-displayed CTB receptors were evaluated. It could be concluded that the **chimeric** receptors were **targeted** to the cell wall of the staphylococci, since they could be released by lysostaphin treatment and, after subsequent affinity purifn., identified as full-length products by immunoblotting. Surface accessibility of the **chimeric** receptors was demonstrated by a colorimetric assay and by immunofluorescence staining with a CTB-reactive rabbit antiserum. Pentamerization was investigated by using a monoclonal antibody described to be specific for pentameric CTB, and the functionality of the receptors was tested in a **binding** assay with digoxigenin-labeled GM1. It was concluded that functional CTB was present on both types of staphylococci, and for *S. carnosus*, the reactivity to the pentamer-specific monoclonal antibody and in the GM1 **binding** assay was indeed significant. The implications of the results for the design of live bacterial vaccine delivery systems

intended

for administration by the mucosal route are discussed.

L7 ANSWER 7 OF 17 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1996:394305 CAPLUS

DOCUMENT NUMBER: 125:106492

TITLE: The import receptor for the peroxisomal **targeting** signal 2 (PTS2) in *Saccharomyces cerevisiae* is encoded by the PAS7 gene

AUTHOR(S): Rehling, peter; Marzioch, Martina; Niesen, Frank; Wittke, Evelyn; Veenhuis, Marten; Kunau, Wolf-H. Inst. Physiologische Chemie, Ruhr-Univ. Bochum, Bochum, D-44780, Germany

SOURCE: EMBO J. (1996), 15(12), 2901-2913

CODEN: EMJODG; ISSN: 0261-4189

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The import of peroxisomal matrix proteins is dependent on one of two **targeting** signals, PTS1 and PTS2. We demonstrate in vivo that not only the import of thiolase but also that of a **chimeric protein** consisting of the thiolase PTS2 (amino acids 1-18) fused to the bacterial **protein .beta.-lactamase** is Pas7p dependent. In addn., using a combination of several independent approaches (two-hybrid system, co-immunopptn., affinity chromatog. and high copy suppression), we show that Pas7p specifically interacts with thiolase in vivo and in vitro. For this interaction, the N-terminal PTS2 of thiolase is both necessary and sufficient. The specific **binding** of Pas7p to thiolase does not require peroxisomes. Pas7p recognizes the PTS2 of thiolase even when this otherwise N-terminal **targeting** signal is fused to the C-terminus of other proteins, i.e. the activation domain of Gal4p or GST. These results demonstrate that Pas7p is the **targeting** signal-specific receptor of thiolase in *Saccharomyces cerevisiae* and, moreover, are consistent with the view that Pas7p is the general receptor of the PTS2. Our observation that Pas7p also interacts with the human peroxisomal thiolase suggests that in the human peroxisomal disorders characterized by an import defect for PTS2 proteins (classical rhizomelic chondrodysplasia punctata), a

functional homolog of Pas7p may be impaired.

L7 ANSWER 8 OF 17 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1995:372817 CAPLUS

DOCUMENT NUMBER: 122:181411

TITLE: A novel cellulose **binding** domain with high affinity for cellulose and chitin and its

applications

INVENTOR(S): Shoseyov, Oded; Shpiegl, Itai; Goldstein, Marc A.; Doi, Roy H.

PATENT ASSIGNEE(S): Regents of the University of California, USA; Yissum Research Development Co.

SOURCE: PCT Int. Appl., 125 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9424158	A1	19941027	WO 94-US4132	19940414
W: AU, BB, BG, BR, BY, CA, CN, CZ, FI, GE, HU, JP, KG, KR, KZ, LK, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, UA, US,				
U2				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 5496934	A	19960305	US 93-48164	19930414
CA 2160670	AA	19941027	CA 94-2160670	19940414
AU 9466347	A1	19941108	AU 94-66347	19940414
AU 691807	B2	19980528		
EP 695311	A1	19960207	EP 94-914175	19940414
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT,				
SE				
CN 1125452	A	19960626	CN 94-192440	19940414
JP 08509127	T2	19961001	JP 94-523460	19940414
US 5670623	A	19970923	US 95-460462	19950602
US 5719044	A	19980217	US 95-460457	19950602
US 5738984	A	19980414	US 95-460458	19950602
US 5837814	A	19981117	US 95-460455	19950602
NO 9504074	A	19951127	NO 95-4074	19951013
FI 9504888	A	19951213	FI 95-4888	19951013
PRIORITY APPLN. INFO.:			US 93-48164	19930414
			WO 94-US4132	19940414

AB A novel cellulose **binding** domain (CBD) with a high affinity for
cryst. cellulose and chitin is described and a DNA sequence encoding it
is

cloned and expressed. Fusion products of the CBD and a second **protein** are described and have a wide range of applications including drug delivery, affinity sepns., and diagnostic techniques. The CBD in this case is derived from cellulose-**binding protein** A of Clostridium cellulovorans. The coding sequence for the carbohydrate-**binding** domain was cloned by PCR and expressed in Escherichia coli. Characterization of the CBD is described. A **chimeric** gene for a fusion **protein** of the CBD and **Protein A** was constructed and expressed in Escherichia coli. The fusion **protein** was purified in a single step affinity chromatog. against cellulose. This complex could bind IgG and the IgG could be eluted from the complex by acetic acid 1 M without elution of the fusion

protein.

L7 ANSWER 9 OF 17 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1995:285392 CAPLUS

DOCUMENT NUMBER: 122:64160

TITLE: Development of a humanized disulfide-stabilized anti-p185HER2 Fv-**.beta.-lactamase** fusion **protein** for activation of a cephalosporin doxorubicin prodrug

AUTHOR(S): Rodriguez, Maria L.; Presta, Leonard G.; Kotts, Claire

E.; Wirth, Cindy; Mordenti, Joyce; Osaka, Gary; Wong, Wai Lee T.; Nuijens, Andrew; Blackburn, Brent;

Carter,

Paul

CORPORATE SOURCE: Departments of Cell Genetics, Genentech Inc., South San Francisco, CA, 94080-4990, USA

SOURCE: Cancer Res. (1995), 55(1), 63-70

CODEN: CNREA8; ISSN: 0008-5472

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The humanized anti-p185HER2 antibody, humAb4D5-8, has completed Phase II clin. trials for p185HER2 overexpressing breast cancer. Here, this antibody is used as a building block to engineer a disulfide-linked Fv (dsFv) **.beta.-lactamase** fusion **protein** for use in antibody-dependent **enzyme**-mediated prodrug therapy using cephalosporin-based prodrugs. Three Fv variants were designed with an interchain disulfide bond buried at the VL/VH interface and secreted from *Escherichia coli*. One **variant**, dsFv3 (VLL46C VH D101C), has similar affinity for antigen (Kd = 0.7 nM) as the wild-type Fv and was used to construct a fusion **protein** in which **.beta.-lactamase**, RTEM-1, is joined to the carboxy terminus of VH. The dsFv3-**.beta.-lactamase** fusion **protein** secreted from *E. coli* efficiently activates a cephalothin doxorubicin prodrug (PRODOX, kcat/km = 1.5 .times. 105 s⁻¹ M⁻¹). PRODOX is approx. 20-fold less toxic than free doxorubicin against breast tumor cell lines SK-BR-3 and MCF7, which express p185HER2 at elevated and normal levels, resp. Prebinding the dsFv3-**.beta.-lactamase** fusion **protein** specifically enhances the toxicity level of PRODOX to that of doxorubicin against SK-BR-3 but not MCF7 cells. The fusion **protein** retains both antigen-**binding** plus kinetic activity in murine serum and is cleared rapidly as judged by pharmacokinetic anal. in nude mice (initial and terminal half-lives of 0.23 and 1.27 h, resp.). Development and characterization of the dsFv3-**.beta.-lactamase** fusion **protein** is an important step toward **targeted** prodrug therapy of p185HER2-overexpressing tumors.

L7 ANSWER 10 OF 17 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1994:426877 CAPLUS

DOCUMENT NUMBER: 121:26877

TITLE: Activation of prodrugs by enzymes that are fusion products of catalytic domains and antigen-**binding** domains

INVENTOR(S): Gehrman, Mathias; Seemann, Gerhard; Bosslet, Klaus; Czech, Joerg

PATENT ASSIGNEE(S): Behringwerke Aktiengesellschaft, Germany

SOURCE: Eur. Pat. Appl., 35 pp.

DOCUMENT TYPE: Patent
 LANGUAGE: German
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

CODEN: EPXXDW

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 590530	A2	19940406	EP 93-115418	19930924
EP 590530	A3	19970326		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
DE 4233152	A1	19940407	DE 92-4233152	19921002
CA 2107513	AA	19940403	CA 93-2107513	19931001
NO 9303520	A	19940405	NO 93-3520	19931001
ZA 9307299	A	19940425	ZA 93-7299	19931001
AU 9348791	A1	19940414	AU 93-48791	19931004
AU 672431	B2	19961003		
JP 06228195	A2	19940816	JP 93-271291	19931004

PRIORITY APPLN. INFO.:

DE 92-4233152 19921002

AB Activation of prodrugs of cytotoxins at a defined **target** site is achieved using a fusion **protein** of prodrug-activating **enzyme** and an antigen-**binding** domain specific for an antigen of the **target** site. A fusion **protein** of a humanized sFv fragment of an antibody to carcinoembryonic antigen and a human .beta.-glucuronidase was prepd. by expression of the **chimeric** gene in BHK cells. The **protein** was shown to specifically bind CEA and to hydrolyze 4-methyl-umbelliferyl-.beta.-glucuronide. Manuf. of the fusion **protein** in yeast is demonstrated. Pharmacokinetics of the fusion **protein** 0.8 .mu.g injected into tumor-bearing nude mice showed very rapid clearing of the **protein** from the plasma and all other organs. The highest levels of the **protein** were found in the tumor with a concn. of 6.2 ng/g tissue after 120 h compared to <0.1 ng/g for all other tissue tested.

L7 ANSWER 11 OF 17 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1993:510554 CAPLUS

DOCUMENT NUMBER: 119:110554

TITLE: Expression of proteins on gram-negative bacterial surface

INVENTOR(S): Georgiou, George; Francisco, Joseph A.; Earhart, Charles F.

PATENT ASSIGNEE(S): USA

SOURCE: PCT Int. Appl., 69 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9310214	A1	19930527	WO 92-US9756	19921110
W: CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE				
US 5348867	A	19940920	US 91-794731	19911115
EP 746621	A1	19961211	EP 93-909521	19921110
R: BE, CH, DE, FR, GB, LI, NL, SE				

PRIORITY APPLN. INFO.:

US 91-794731 19911115
 WO 92-US9756 19921110

AB Tripartite **chimeric** genes are constructed for expression of proteins on the surface of the gram-neg. bacteria such as Escherichia. The tripartite **chimeric** genes comprise from 5'-end a **targeting** DNA sequence such as a signal sequence and N-terminal amino acids of OmpA gene of E. coli, a transmembrane amino acids-coding sequence such as that of the outer membrane **protein** of E. coli, and the gene of interest. Plasmid pTX101 contg. a tripartite **chimeric** genes are constructed for expression of **protein** for signal sequence and the first 9 amino acids of OmpA **protein**, the transmembrane amino acids of the same **protein**, and . **beta.-lactamase** (I) was constructed from pJG311 and pRD87. The plasmid was transformed into E.coli JM109; 84% of total I activity was found in the cell envelope fraction; detd. by immunocytochem methods, the I remained stably anchored on the surface of the cell even after extended incubation. Also given was the cloning of antibodies with high antigen **binding** affinity by expression of the antibodies on the surface of the cells.

L7 ANSWER 12 OF 17 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1993:118249 CAPLUS

DOCUMENT NUMBER: 118:118249

TITLE: Enrichment method for **variant** proteins with altered **binding** properties

INVENTOR(S): Garrard, Lisa J.; Henner, Dennis J.; Bass, Steven; Greene, Roland; Lowman, Henry B.; Wells, James A.; Matthews, David J.

PATENT ASSIGNEE(S): Genentech, Inc., USA

SOURCE: PCT Int. Appl., 101 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9209690	A2	19920611	WO 91-US9133	19911203
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE				
CA 2095633	AA	19920604	CA 91-2095633	19911203
EP 564531	A1	19931013	EP 92-902109	19911203
EP 564531	B1	19980325		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL, SE				
JP 07503600	T2	19950420	JP 91-502710	19911203
AT 164395	E	19980415	AT 92-902109	19911203
ES 2113940	T3	19980516	ES 92-902109	19911203
US 5750373	A	19980512	US 93-50058	19930430
US 5688666	A	19971118	US 94-182530	19940114
US 5780279	A	19980714	US 95-418928	19950405
US 5846765	A	19981208	US 95-441871	19950516
PRIORITY APPLN. INFO.:			US 90-621667	19901203
			US 91-683400	19910410
			US 91-715300	19910614
			US 91-743614	19910808
			US 88-264611	19881028
			US 91-682400	19910410
			WO 91-US9133	19911203
			US 92-864452	19920419
			US 93-50058	19930430

US 93-161692 19931203
US 95-418928 19950405

AB A method for selecting variants of proteins such as growth hormone and antibody fragment with altered **binding** properties for their resp. receptor mols. is provided. The method comprises fusing a gene encoding a **protein** of interest to at least a portion of the gene for a phage coat **protein**, e.g. for the C-terminal domain of the gene III coat **protein** of M13 under control of a transcription-regulating element. The vector is mutated at .gtoreq.1 position within the 1st gene (e.g. by oligonucleotide-directed mutagenesis), and host cells are transformed with the mutant vector and a helper phage having the coat **protein** gene. Recombinant phagemid particles are formed contg. at least part of the mutant expression vector and capable of transforming the host; conditions are adjusted so that

most

phagemid particles do not display >1 copy of the fusion **protein** on the particle surface. The phagemid particles are screened for **binding** to the **target** mol. These steps may be repeated. Phagemids presenting human growth hormone (hGH)-gene III **protein** fusion proteins prep'd. as above were fractionated chromatog. on immobilized hGH-**binding protein**; a single cycle of **binding** and elution gave >5000-fold enrichment.

L7 ANSWER 13 OF 17 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1993:33872 CAPLUS

DOCUMENT NUMBER: 118:33872

TITLE: Metalloregulated expression of the ars operon

AUTHOR(S): Wu, Jianhua; Rosen, Barry P.

CORPORATE SOURCE: Sch. Med., Wayne State Univ., Detroit, MI, 48201, USA

SOURCE: J. Biol. Chem. (1993), 268(1), 52-8

CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The plasmid-borne arsenical resistance (ars) operon encodes an arsenical-translocating ATPase and confers resistance to antimonials and arsenicals in Escherichia coli by extrusion of the toxic compds. from the cytosol. The trans-acting regulatory ArsR **protein** was shown to bind to a fragment of DNA contg. the ars promoter. Hybrid formation of the ArsR **protein** with a ArsR-**beta**-lactamase chimeric **protein** suggested that the active form of the ArsR repressor is a dimer. From footprinting anal. the **binding** site was defined as a region of imperfect dyad symmetry just upstream of the -35 site. In vivo the operon was derepressed by oxyions of +III oxidn. state of arsenic, antimony, and bismuth, as well as arsenate (As(V)), whereas in vitro ArsR **protein**-operator interaction was reduced by each of those compds. except arsenate, as det'd. by gel retardation and DNase I and hydroxyl radical footprinting expts. This indicates that arsenate is not a true inducer and must be reduced to arsenite in vivo to induce. An operator mutant obtained by deletion of the in vitro ArsR-protected DNA sequence exhibited constitutive ars promoter activity, demonstrating that the **binding** site is the functional **target** for the ArsR repressor in vivo.

L7 ANSWER 14 OF 17 BIOSIS COPYRIGHT 1999 BIOSIS

ACCESSION NUMBER: 1996:361772 BIOSIS

DOCUMENT NUMBER: PREV199699084128

TITLE: The import receptor for the peroxisomal **targeting** signal 2 (PTS2) in Saccharomyces cerevisiae is encoded by

the PAS7 gene.
AUTHOR(S): Rehling, Peter; Marzioch, Martina; Niesen, Frank; Wittke, Evelyn; Veenhuis, Marten; Kunau, Wolf-H. (1)
CORPORATE SOURCE: (1) Inst. Physiol. Chem., Ruhr-Univ Bochum, Medizin. Fak., Abt. Zellbiochem., D-44780 Bochum Germany
SOURCE: EMBO (European Molecular Biology Organization) Journal, (1996) Vol. 15, No. 12, pp. 2901-2913.
ISSN: 0261-4189.

DOCUMENT TYPE: Article
LANGUAGE: English

AB The import of peroxisomal matrix proteins is dependent on one of two **targeting** signals, PTS1 and PTS2. We demonstrate in vivo that not only the import of thiolase but also that of a **chimeric protein** consisting of the thiolase PTS2 (amino acids 1-18) fused to the bacterial **protein beta-lactamase** is Pas7p dependent. In addition, using a combination of several independent approaches (two-hybrid system, co-immunoprecipitation, affinity chromatography and high copy suppression), we show that Pas7p specifically interacts with thiolase in vivo and in vitro. For this interaction, the N-terminal PTS2 of thiolase is both necessary and sufficient. The specific **binding** of Pas7p to thiolase does not require peroxisomes. Pas7p recognizes the PTS2 of thiolase even when this otherwise N-terminal **targeting** signal is fused to the C-terminus of other proteins, i.e. the activation domain of Gal4p or GST. These results demonstrate that Pas7p is the **targeting** signal-specific receptor of thiolase in *Saccharomyces cerevisiae* and, moreover, are consistent with the view that Pas7p is the general receptor of the PTS2. Our observation that Pas7p also interacts with the human peroxisomal thiolase suggests that in the human peroxisomal disorders characterized by an import defect for PTS2 proteins (classical rhizomelic chondrodysplasia punctata), a functional homologue of Pas7p may be impaired.

L7 ANSWER 15 OF 17 BIOSIS COPYRIGHT 1999 BIOSIS

ACCESSION NUMBER: 1995:111962 BIOSIS

DOCUMENT NUMBER: PREV199598126262

TITLE: Development of a humanized disulfide-stabilized anti-p185-HER2 Fv-**beta-lactamase** fusion **protein** for activation of a cephalosporin doxorubicin prodrug.

AUTHOR(S): Rodrigues, Maria L.; Presta, Leonard G.; Kotts, Claire E.; Wirth, Cindy; Mordenti, Joyce; Osaka, Gary; Wong, Wai Lee T.; Nuijens, Andrew; Blackburn, Brent; Carter, Paul (1)

CORPORATE SOURCE: (1) Genentech Inc., Dep. Cell Genetics, 460 Point San Bruno

Boulevard, South San Francisco, CA 94080-4900 USA
SOURCE: Cancer Research, (1995) Vol. 55, No. 1, pp. 63-70.
ISSN: 0008-5472.

DOCUMENT TYPE: Article
LANGUAGE: English

AB The humanized anti-p185-HER2 antibody, humAb4D5-8, has completed Phase II clinical trials for p185-HER2-overexpressing breast cancer. Here, this antibody is used as a building block to engineer a disulfide-linked Fv (dsFv) **beta-lactamase** fusion **protein** for use in antibody-dependent **enzyme**-mediated prodrug therapy using cephalosporin-based prodrugs. Three Fv variants were designed with an

interchain disulfide bond buried at the V-L/V-H interface and secreted from *Escherichia coli*. One **variant**, dsFv3 (V-L L46C V-H D101C), has similar affinity for antigen (K-d = 0.7 nm) as the wild-type Fv and was used to construct a fusion **protein** in which **beta-lactamase**, RTEM-1, is joined to the carboxy terminus of V-H. The dsFv3-**beta-lactamase** fusion **protein** secreted from *E. coli* efficiently activates a cephalothin doxorubicin prodrug (PRODOX, k-cat/k-m = 1.5 times 10⁻⁵ s⁻¹ M⁻¹). PRODOX is approximately 20-fold less toxic than free doxorubicin against breast tumor cell lines SK-BR-3 and MCF7, which express p185-HER2 at elevated and normal levels, respectively. Prebinding the dsFv3-**beta-lactamase** fusion **protein** specifically enhances the toxicity level of PRODOX to that of doxorubicin against SK-BR-3 but not MCF7 cells. The fusion **protein** retains both antigen-**binding** plus kinetic activity in murine serum and is cleared rapidly as judged by pharmacokinetic analysis in nude mice (initial and terminal half-lives of 0.23 and 1.27 h, respectively). Development and characterization of the dsFv3-**beta-lactamase** fusion **protein** is an important step toward **targeted** prodrug therapy of p185-HER2-overexpressing tumors.

L7 ANSWER 16 OF 17 BIOSIS COPYRIGHT 1999 BIOSIS

ACCESSION NUMBER: 1993:117416 BIOSIS

DOCUMENT NUMBER: PREV199395061516

TITLE: Metalloregulated expression of the ars operon.

AUTHOR(S): Wu, Jianhua; Rosen, Barry P. (1)

CORPORATE SOURCE: (1) Dep. Biochem., Wayne State Univ. Sch. Med., 540 E. Canfield Ave., Detroit, Mich. 48201

SOURCE: Journal of Biological Chemistry, (1993) Vol. 268, No. 1, pp. 52-58.
ISSN: 0021-9258.

DOCUMENT TYPE: Article

LANGUAGE: English

AB The plasmid-borne arsenical resistance (ars) operon encodes an arsenical-translocating ATPase and confers resistance to antimonials and arsenicals in *Escherichia coli* by extrusion of the toxic compounds from the cytosol. The trans-acting regulatory ArsR **protein** was shown to bind to a fragment of DNA containing the ars promoter. Hybrid formation of the ArsR **protein** with a ArsR-**beta-lactamase** **chimeric protein** suggested that the active form of the ArsR repressor is a dimer. From footprinting analysis the **binding** site was defined as a region of imperfect dyad symmetry just upstream of the -35 site. In vivo the operon was derepressed by oxyions of +III oxidation state of arsenic, antimony, and bismuth, as well as arsenate (As(V)), whereas in vitro ArsR **protein**-operator interaction was reduced by each of those compounds except arsenate, as determined by gel retardation and DNase I and hydroxyl radical footprinting experiments. This indicates that arsenate is not a true inducer and must be reduced to arsenite in vivo to induce. An operator mutant obtained by deletion of the in vitro ArsR-protected DNA sequence exhibited constitutive ars promoter activity, demonstrating that the **binding** site is the functional **target** for the ArsR repressor in vivo.

L7 ANSWER 17 OF 17 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD

ACCESSION NUMBER: 93-182531 [22] WPIDS

DOC. NO. CPI: C93-080879

TITLE: New tripartite **chimeric** gene and related recombinant vector - for expressing proteins e.g. **beta-lactamase**, alkaline phosphatase, on the surface of gram-negative bacteria.

DERWENT CLASS: B04 D16

INVENTOR(S): EARHART, C F; FRANCISCO, J A; GEORGIU, G

PATENT ASSIGNEE(S): (GEOR-I) GEORGIU G

COUNTRY COUNT: 9

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9310214	A1	930527	(9322)*	EN	66
US 5348867	A	940920	(9437)		26
EP 746621	A1	961211	(9703)	EN	

R: BE CH DE FR GB LI NL SE

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9310214	A1	WO 92-US9756	921110
US 5348867	A	US 91-794731	911115
EP 746621	A1	WO 92-US9756	921110
		EP 93-909521	921110

FILING DETAILS:

PATENT NO	KIND	PATENT NO
EP 746621	A1 Based on	WO 9310214

PRIORITY APPLN. INFO: US 91-794731 911115

AB WO 9310214 A UPAB: 931115

The tripartite **chimeric** gene comprises: (a) a **targetting** DNA sequence encoding a polypeptide capable of **targetting** a fusion polypeptide to a gram-negative cell over membrane; (b) a DNA segment encoding a transmembrane aminoacid sequence capable of transporting a heterologous or homologous polypeptide through a gram-negative outer membrane; and (c) at least one DNA segment encoding a heterologous or sol. homologous polypeptide where the **chimeric** gene is expressible in a gram negative bacterial cell when preceded by a functional promoter. Alternatively the recombinant vector capable of expressing a heterologous or soluble polypeptide on a gram-negative bacterium outer membrane external surface. (a) a **targetting** DNA sequence encoding a polypeptide capable of **targetting** to the outer membrane of a gram negative bacterial cell; (b) a transmembrane DNA sequence positioned adjacent to the **targetting** DNA sequence, the transmembrane sequence encoding a polypeptide capable of transvering the cell outer membrane; and (c) a DNA sequence encoding a heterologous or soluble homologous polypeptide, the sequence positioned adjacent to the transmembrane sequence.

USE/ADVANTAGE - Numerous types of fusion polypeptides may be expressed by the system of (A). The tripartile **chimeric** gene and its related recombinant vector include separate DNA sequences for directing or **targetting** and translocating a desired gene prod. from a cell periplasm to the external cell surface. A wide range of polypeptides may be efficiently surface expressed, including **beta**

-lactamase, alkaline phosphatase, cellulose binding domain of cellulose, or single chain; Fv antibody. Full enzyme activity is maintained and the proteins remain anchored to the bacterial outer membrane surface. Expression of single-chain antibodies on the surface of gram-negative bacterial hosts has several potential applications, esp. for prepn. of whole cell adsorbants. A variety of antigenic determinants may be expressed on a cell surface and used to prepare bacterial vaccines. A selected antigen in combination with an activating agent e.g. IL-4 on the surface of the bacterium may have potential use in stimulating an immune response towards a surface exposed antigen.

Dwg.0/9

=> s (chimeric enzyme? or variant enzyme?) and beta lactamase and mimetope

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4525 CHIMERIC
68485 ENZYME?
23 CHIMERIC ENZYME?
(CHIMERIC(W) ENZYME?)
50495 VARIANT
68485 ENZYME?
47 VARIANT ENZYME?
(VARIANT(W) ENZYME?)
178933 BETA
3171 LACTAMASE
3011 BETA LACTAMASE
(BETA(W) LACTAMASE)
32 MIMETOPE
L1 0 (CHIMERIC ENZYME? OR VARIANT ENZYME?) AND BETA LACTAMASE AN
D M
IMETOPE
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=> s (chimeric enzyme? or variant enzyme?) and beta lactamase

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4525 CHIMERIC
68485 ENZYME?
23 CHIMERIC ENZYME?
(CHIMERIC(W) ENZYME?)
50495 VARIANT
68485 ENZYME?
47 VARIANT ENZYME?
(VARIANT(W) ENZYME?)
178933 BETA
3171 LACTAMASE
3011 BETA LACTAMASE
(BETA(W) LACTAMASE)
L2 18 (CHIMERIC ENZYME? OR VARIANT ENZYME?) AND BETA LACTAMASE
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=> d 12 1-18

1. 5,866,775, Feb. 2, 1999, Glyphosate-tolerant 5-enolpyruvyl-3-phosphoshikimate synthases; David Alan Eichholtz, et al., 47/DIG.1; 435/320.1, 419; 536/23.2, 23.6, 23.7 [IMAGE AVAILABLE]
2. 5,846,765, Dec. 8, 1998, Identification of novel substrates; David J. Matthews, et al., 435/69.1, 7.2, 7.6, 69.7, 320.1 [IMAGE AVAILABLE]
3. 5,837,517, Nov. 17, 1998, Protease variants and compositions; Laurens Nicolaas Sierkstra, et al., 435/221, 69.1, 222, 252.3, 252.31, 320.1; 510/300, 320; 536/23.2 [IMAGE AVAILABLE]
4. 5,834,250, Nov. 10, 1998, Method for identifying active domains and amino acid residues in polypeptides and hormone variants; James A. Wells, et al., 435/7.1, 4, 6; 436/501; 530/300, 326 [IMAGE AVAILABLE]
5. 5,804,425, Sep. 8, 1998, Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases; Gerard Francis Barry, et al., 435/193, 252.3, 252.33, 320.1, 822, 839, 874, 883 [IMAGE AVAILABLE]
6. 5,780,279, Jul. 14, 1998, Method of selection of proteolytic cleavage sites by directed evolution and phagemid display; David J. Matthews, et al., 435/6, 69.1, 243, 320.1, 325, 472, 488; 530/300, 399 [IMAGE AVAILABLE]

AVAILABLE]

7. 5,679,548, Oct. 21, 1997, Methods for producing polypeptide metal binding sites and compositions thereof; Carlos F. Barbas, et al., 435/69.6, 70.21, 188.5, 477; 514/6; 530/387.1, 400; 536/23.53, 24.3; 930/25 [IMAGE AVAILABLE]
8. 5,652,136, Jul. 29, 1997, Substrate assisted catalysis; Paul John Carter, et al., 435/252.3, 221, 222, 320.1; 536/23.4 [IMAGE AVAILABLE]
9. 5,633,435, May 27, 1997, Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases; Gerard F. Barry, et al., 800/288; 435/320.1, 411, 413, 414, 415, 416, 417, 418; 504/206; 536/23.2; 800/294, 300, 300.1, 312 [IMAGE AVAILABLE]
10. 5,627,061, May 6, 1997, Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases; Gerard F. Barry, et al., 800/288; 435/320.1 [IMAGE AVAILABLE]
11. 5,599,670, Feb. 4, 1997, .beta.-glucuronidase and glucuronide permease gene system; Richard A. Jefferson, 435/6, 183, 200, 252.3, 252.33, 320.1, 325, 418, 419, 455, 468, 469, 476; 514/44; 536/23.2, 23.7, 24.1 [IMAGE AVAILABLE]
12. 5,580,723, Dec. 3, 1996, Method for identifying active domains and amino acid residues in polypeptides and hormone variants; James A. Wells, et al., 435/6, 71.1, 69.1, 71.1; 436/501; 530/387.1, 388.1, 399, 806, 808 [IMAGE AVAILABLE]
13. 5,472,855, Dec. 5, 1995, Substrate assisted catalysis; Paul J. Carter, et al., 435/68.1, 221, 222 [IMAGE AVAILABLE]
14. 5,371,190, Dec. 6, 1994, Substrate assisted catalysis; Paul J. Carter, et al., 530/350; 435/221, 222 [IMAGE AVAILABLE]
15. 5,371,008, Dec. 6, 1994, Substrate assisted catalysis; Paul J. Carter, et al., 435/222, 221; 530/358 [IMAGE AVAILABLE]
16. 5,366,729, Nov. 22, 1994, Non-glycosylated variants of extracellular superoxide dismutase (EC-SOD); Stefan Marklund, et al., 424/94.4; 435/189, 320.1, 325, 358; 536/23.2 [IMAGE AVAILABLE]
17. 5,310,667, May 10, 1994, Glyphosate-tolerant 5-enolpyruvyl-3-phosphoshikimate synthases; David A. Eichholtz, et al., 435/91.1, 69.1; 536/23.6, 23.7 [IMAGE AVAILABLE]
18. 5,268,463, Dec. 7, 1993, Plant promoter .alpha.-glucuronidase gene construct; Richard A. Jefferson, 536/23.7; 435/200, 320.1; 536/24.1 [IMAGE AVAILABLE]

=> l1 kwic

L2 18 S (CHIMERIC ENZYME? OR VARIANT ENZYME?) AND BETA LACTAMASE

=> d kwic l2

DETDESC:

DETD(30)

Variant . . . for PEP, normal catalytic activity will only be achieved at physiological concentrations of PEP by an elevated level of the ****variant** **enzyme****. If a variant of EPSP synthase could be identified that had a high K.sub.i for glyphosate and a lower K.sub.m. .

DETDESC:

DETD(40)

Plasmid . . . cleaved with ClaI. The resulting plasmid, pMON8, contains the EPSP synthase coding sequence reading in the same direction as the ****beta**--**lactamase**** gene of pBR327.

DETDESC:

DETD(62)

The weakly expressing pMON8135 (FIG. 2) construct containing the known ****variant** **enzyme**** sequence was not able to complement the *aroA* defect in SR481 and did not support cell growth on minimal medium. . . a specific activity similar to its parental construct pMON8105. It was then hypothesized that the elevated K.sub.m for PEP of the ****variant** **enzyme**** (198 .mu.M versus 5.2 .mu.M for the wild type) resulted in a relatively inefficient EPSP synthase enzyme that was unable. . . and the ability of a variant petunia EPSPS enzyme to complement *aroA* when weakly expressed in *E. coli*. If a ****variant** **enzyme**** has a high K.sub.m for PEP, then a greater level of expression is required to complement *aroA*. The weakly expressing. . .

DETDESC:

DETD(84)

The . . . 2000 .mu.M and a K.sub.m for PEP of 200 .mu.M. The K.sub.i /K.m ratio for the pMON8252 encoded glyphosate resistant ****variant** **enzyme**** is 12.6, which is similar to that of the progenitor glycine (101) to alanine variant whose ratio is 10.0. However, . . . that is four fold lower than the glycine (101) to alanine variant. The lowering of the K.sub.m for PEP makes the pMON8252 ****variant** **enzyme**** more efficient kinetically, as demonstrated by its ability to support the growth of *E. coli* even when expressed with the. . . demonstrated that the selection system allowed for the induction and identification of mutations of the petunia EPSPS glycine (101) to alanine ****variant** **enzyme**** which would maintain the glyphosate resistant properties of the original variant, but lower the K.sub.m for PEP. The pMON8252 results. . .

DETDESC:

DETD(86)

To . . . codon, GAA also codes for glutamic acid. Thus, the improved kinetic properties of the pMON8252 encoded glyphosate resistant petunia

EPSPS **variant** **enzyme** are due to a combination of two substitutions: one resulting in the glycine (101) to alanine change, the other resulting. . .

=> d 12 2 4 12 ab ti clm

US PAT NO: 5,846,765 [IMAGE AVAILABLE]

L2: 2 of 18

ABSTRACT:

A method for identifying and selecting novel substrates for enzymes is provided. The method comprises constructing a gene fusion comprising DNA encoding a polypeptide fused to DNA encoding a substrate peptide, which in turn is fused to DNA encoding at least a portion of a phage coat protein. The DNA encoding the substrate peptide is mutated at one or more codons thereby generating a family of mutants. The fusion protein is expressed on the surface of a phagemid particle and subjected to chemical or enzymatic modification of the substrate peptide. Those phagemid particles which have been modified are then separated from those that have not.

TITLE: Identification of novel substrates

CLAIMS:

CLMS(1)

We claim:

1. A method for selecting novel polypeptide substrates comprising:
 - (a) constructing a replicable expression vector comprising a transcription regulatory element operably linked to a gene fusion, wherein the gene fusion comprises:
 - (i) a first gene encoding a polypeptide;
 - (ii) a second gene encoding a substrate peptide; and
 - (iii) a third gene encoding at least a portion of a phage coat protein, wherein the 3' end of the first gene is linked to the 5' end of the second gene, and the 3' end of the second gene is linked to the 5' end of the third gene;
 - (b) mutating the vector at one or more selected positions within the second gene thereby forming a family of related plasmids encoding substrate peptides;
 - (c) transforming suitable host cells with the plasmids;
 - (d) infecting the transformed host cells with a helper phage having a gene encoding the phage coat protein;
 - (e) culturing the transformed infected host cells under conditions suitable for forming recombinant phagemid particles containing at least a portion of the plasmid and capable of transforming the host, the conditions adjusted so that no more than a minor amount of phagemid particles display more than one copy of the fusion protein on the surface of the particle;
 - (f) exposing the phagemid particles to a process capable of modifying at least one covalent bond of an amino acid in the substrate peptide of at least a portion of the phagemid particles;
 - (g) contacting the family of exposed phagemid particles with an affinity molecule, wherein the affinity molecule has affinity for the amino acid residue having the modified covalent bond; and
 - (h) separating the phagemid particles that bind to the affinity molecule from those that do not.

CLMS(2)

2. The method of claim 1 wherein the process comprises a post-translational covalent bond modifying process selected from the group consisting of a phosphorylation, glycosylation, carboxylation, ADP-ribosylation, methylation, isoprenylation and acylation.

CLMS(3)

3. The method of claim 2 wherein the process comprises phosphorylation of an amino acid selected from Thr, Ser, and Tyr.

CLMS(4)

4. The method of claim 2 wherein the affinity molecule is a monoclonal antibody having specificity for the modified amino acid of the process.

CLMS(5)

5. The method of claim 2 wherein the phagemid particles that bind the affinity molecule are eluted, infected into suitable host cells, and steps (d) through (h) are repeated two or more times.

CLMS(6)

6. The method of claim 2 wherein the phagemid particles that do not bind to the affinity molecule are infected into suitable host cells and steps (d) through (h) are repeated two or more times.

CLMS(7)

7. A method for selecting novel polypeptides substrates comprising:
(a) constructing a replicable expression vector comprising a transcription regulatory element operably linked to a gene fusion, wherein the gene fusion comprises:
 (i) a first gene encoding a polypeptide;
 (ii) a second gene encoding a substrate peptide; and
 (iii) a third gene encoding at least a portion of a phage coat protein, wherein the 3' end of the first gene is linked to the 5' end of the second gene, and the 3' end of the second gene is linked to the 5' end of the third gene;
(b) mutating the vector at one or more selected positions within the second gene thereby forming a family of related plasmids encoding substrate peptides;
(c) transforming suitable host cells with the plasmids;
(d) infecting the transformed host cells with a helper phage having a gene encoding the phage coat protein;
(e) culturing the transformed infected host cells under conditions suitable for forming recombinant phagemid particles containing at least a portion of the plasmid and capable of transforming the host, the conditions adjusted so that no more than a minor amount of phagemid particles display more than one copy of the fusion protein on the surface of the particle;
(f) exposing the phagemid particles to a process capable of modifying at least one covalent bond of an amino acid residue in the substrate peptide of at least a portion of the phagemid particles;
(g) derivatizing the modified amino acid residue with a substituent capable of binding with an affinity molecule;
(h) contacting the family of exposed derivitized particles with an affinity molecule, wherein the affinity molecule has affinity for the substituent; and

(1) separating the phagemid particle that bind to the affinity molecule from those that do not.

CLMS(8)

8. The method of claim 7 wherein the process comprises a post-translational covalent bond modifying process selected from the group consisting of a phosphorylation, glycosylation, carboxylation, ADP-ribosylation, methylation, isoprenylation and acylation.

CLMS(9)

9. The method of claim 8 wherein the process comprises phosphorylation of an amino acid selected from Thr, Ser, and Tyr.

CLMS(10)

10. The method of claim 8 wherein the process comprises glycosylation of an amino acid selected from Asn, Ser, and Thr.

CLMS(11)

11. The method of claim 8 wherein the phagemid particles that bind the affinity molecule are eluted, infected into suitable host cells, and steps (d) through (h) are repeated two or more times.

CLMS(12)

12. The method of claim 8 wherein the phagemid particles that do not bind to the affinity molecule are infected into suitable host cells and steps (d) through (h) are repeated two or more times.

CLMS(13)

13. A method for selecting novel polypeptides substrates, comprising:
(a) constructing a family of replicable expression vectors comprising a transcription regulatory element operably linked to a gene fusion, wherein the gene fusion comprises a gene encoding a substrate peptide and a gene encoding at least a portion of a phage coat protein, wherein the 3' end of the gene encoding the substrate peptide is linked to the 5' end of the gene encoding at least a portion of the phage coat protein;
(b) transforming suitable host cells with the replicable expression vectors;
(c) infecting the transformed host cells with a helper phage having a gene encoding the phage coat protein;
(d) culturing the transformed infected host cells under conditions suitable for forming recombinant phagemid particles containing at least a portion of the replicable expression vector and capable of transforming the host cells, the conditions adjusted so that no more than a minor amount of phagemid particles display more than one copy of the fusion protein on the surface of the particles;
(e) exposing the phagemid particles to a process capable of modifying at least one covalent bond of an amino acid in the substrate peptide of at least a portion of the phagemid particles;
(f) contacting the family of exposed phagemid particles with an affinity molecule, wherein the affinity molecule has affinity for the amino acid residue having the modified covalent bond; and
(g) separating the phagemid particles that bind to the affinity molecule from those that do not bind.

CLMS(14)

14. A method for selecting novel polypeptides substrates, comprising:
- (a) constructing a family of replicable expression vectors comprising a transcription regulatory element operably linked to a gene fusion, wherein the gene fusion comprises a gene encoding a substrate peptide and a gene encoding at least a portion of a phage coat protein, wherein the 3' end of the gene encoding the substrate peptide is linked to the 5' end of the gene encoding at least a portion of the phage coat protein;
 - (b) transforming suitable host cells with the replicable expression vectors;
 - (c) infecting the transformed host cells with a helper phage having a gene encoding the phage coat protein;
 - (d) culturing the transformed infected host cells under conditions suitable for forming recombinant phagemid particles containing at least a portion of the replicable expression vector and capable of transforming the host cells, these conditions adjusted so that no more than a minor amount of phagemid particles display more than one copy of the fusion protein on the surface of the particles;
 - (e) exposing the phagemid particles to a process capable of modifying at least one covalent bond of an amino acid residue in the substrate peptide of at least a portion of the phagemid particles;
 - (f) derivatizing the modified amino acid residue with a substituent capable of binding with an affinity molecule;
 - (g) contacting the derivatized particles with an affinity molecule, wherein the affinity molecule has affinity for the substituent; and
 - (h) separating the phagemid particles that bind to the affinity molecule from those that do not bind.

CLMS(15)

15. A method for selecting novel polypeptides, comprising:
- (a) constructing a family of phagemid particles which display different polypeptides fused to at least a portion of a phage coat protein on the surface thereof, wherein no more than a minor amount of phagemid particles display more than one copy of the fusion protein on the surface of the particles;
 - (b) exposing the phagemid particles to a process capable of modifying at least one covalent bond of an amino acid in the polypeptide of at least a portion of the phagemid particles;
 - (c) contacting the family of exposed phagemid particles with an affinity molecule, wherein the affinity molecule has affinity for the amino acid residue having the modified covalent bond; and
 - (d) separating the phagemid particles that bind to the affinity molecule from those that do not bind.

CLMS(16)

16. A method of claim 15, further comprising
- (e) infecting suitable host cells with the phagemid particles that bind or do not bind to the affinity molecule to produce a second family of phagemid particles and repeating steps (b)-(d).

US PAT NO: 5,834,250 [IMAGE AVAILABLE]

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ABSTRACT:

The invention provides methods for the systematic analysis of the structure and function of polypeptides by identifying active domains

which influence the activity of the polypeptide with a target substance. Such active domains are determined by substituting selected amino acid segments of the polypeptide with an analogous polypeptide segment from an analog to the polypeptide. The analog has a different activity with the target substance as compared to the parent polypeptide. The activities of the segment-substituted polypeptides are compared to the same activity for the parent polypeptide for the target. A comparison of such activities provides an indication of the location of the active domain in the parent polypeptide. The invention also provides methods for identifying the active amino acid residues within the active domain of the parent polypeptide. The method comprises substituting a scanning amino acid for one of the amino acid residues within the active domain of the parent polypeptide and assaying the residue-substituted polypeptide so formed with a target substance. The invention further provides polypeptide variants comprising segment-substituted and residue-substituted growth hormones, prolactins and placental lactogens.

TITLE: Method for identifying active domains and amino acid
 residues in polypeptides and hormone variants

CLAIMS:

CLMS (1)

What is claimed is:

1. A method for identifying at least one active amino acid residue in a parent polypeptide which parent polypeptide has an activity resulting from an interaction with a target, said method comprising:
 - (a) substituting a scanning amino acid for a first amino acid residue at residue number N within said parent polypeptide to form an N-substituted polypeptide;
 - (b) substituting a scanning amino acid for each of the amino acid residues at residue numbers N+1 and N-1 to said first residue to form respectively N+1- and N-1-substituted polypeptides;
 - (c) contacting each of said substituted polypeptides with a target to determine the interaction, if any, between said target and said substituted polypeptides;
 - (d) comparing the difference, if any, between the activity of the parent polypeptide and said substituted polypeptides with said target as an indication of the location of said active amino acid residue in said parent polypeptide;
 - (e) repeating steps (b) through (d) for increasing residue numbers if said activity difference between said target and said N+1 substituted polypeptide is greater than two-fold and for decreasing residue numbers if said activity difference between said target and said N-1 substituted polypeptide is greater than two-fold.

CLMS (2)

2. The method of claim 1 wherein steps (b) through (d) are repeated until at least four substituted polypeptides containing the substitution of a scanning amino acid at four consecutive residues are identified having less than a two-fold activity difference as compared to said parent polypeptide.

CLMS (3)

3. A method for identifying at least one active amino acid residue in a polypeptide, comprising the steps of:
 - (a) separately substituting a single scanning amino acid for at least

three amino acid residues in at least one domain of a polypeptide of known amino acid sequence, wherein the polypeptide has a known interaction with a target, to form at least three corresponding substituted polypeptides, each substituted polypeptide containing one scanning amino acid;

- (b) contacting the corresponding substituted polypeptides with the target to determine an interaction, if any, between the target and the corresponding substituted polypeptides; and
- (c) comparing the difference, if any, between the interaction of the target with the polypeptide and the interaction of the target with the corresponding substituted polypeptides as an indication of the location of said active amino acid residue in said polypeptide.

CLMS(4)

4. The method of claim 3, comprising separately substituting a single scanning amino acid for at least 12 amino acid residues in at least one domain of the polypeptide.

CLMS(5)

5. The method of claim 3, comprising separately substituting a single scanning amino acid for at least 18 amino acid residues in at least one domain of the polypeptide.

CLMS(6)

6. The method of claim 5, comprising separately substituting a single scanning amino acid for at least 18-25 amino acid residues in at least one domain of the polypeptide.

CLMS(7)

7. The method of claim 3, comprising separately substituting a single scanning amino acid for each amino acid residue in at least one domain of the polypeptide.

CLMS(8)

8. The method of claim 3, wherein the domain comprises 3-30 amino acid residues.

CLMS(9)

9. The method of claim 8, wherein the domain comprises 3-15 amino acid residues.

CLMS(10)

10. The method of claim 7, wherein the domain comprises at least 12 amino acid residues.

CLMS(11)

11. The method of claim 3, comprising separately substituting the scanning amino acid in two or more domains of a polypeptide.

CLMS(12)

12. The method of claim 3, wherein the single scanning amino acid is a

neutral amino acid.

CLMS(13)

13. The method of claim 12, wherein the neutral amino acid is alanine.

CLMS(14)

14. The method of claim 3, wherein the known interaction is the binding of the target to the polypeptide.

CLMS(15)

15. The method of claim 3, consisting of steps (a), (b), (c).

CLMS(16)

16. A method for identifying at least one active amino acid residue in a polypeptide, comprising the steps of:

- a) separately substituting a single scanning amino acid for each amino acid in a polypeptide of known amino acid sequence containing 12-50 amino acid residues, wherein the polypeptide has a known interaction with a target, to form corresponding substituted polypeptides;
- (b) contacting the corresponding substituted polypeptides with the target to determine an interaction, if any, between the target and the corresponding substituted polypeptides; and
- (c) comparing the difference, if any, between the interaction of the target with the polypeptide and the interaction of the target with the corresponding substituted polypeptides as an indication of the location of said active amino acid residue in said polypeptide.

CLMS(17)

17. The method of claim 16, wherein the polypeptide comprises about 18-50 amino acid residues.

CLMS(18)

18. The method of claim 16, wherein the single scanning amino acid is a neutral amino acid.

CLMS(19)

19. The method of claim 18, wherein the neutral amino acid is selected from the group consisting of alanine, serine, glycine, and cysteine.

CLMS(20)

20. The method of claim 19, wherein the neutral amino acid is alanine.

CLMS(21)

21. The method of claim 16, wherein the known interaction is the binding of the target to the polypeptide.

CLMS(22)

22. The method of claim 16, consisting of steps (a), (b), (c).

CLMS(23)

23. The method of claim 1, wherein the scanning amino acid is selected from the group consisting of Ser, Gly, Gln, Asp, Asn, Glu, Met, Ile, Pro, Arg, Thr, Lys, Val, Tyr and Phe.

CLMS(24)

24. The method of claim 1, wherein the scanning amino acid is selected from the group consisting of alanine, serine, glycine, and cysteine.

CLMS(25)

25. The method of claim 1, wherein the scanning amino acid is alanine.

CLMS(26)

26. The method of claim 1, wherein the scanning amino acid is cysteine.

CLMS(27)

27. The method of claim 3, wherein the scanning amino acid is selected from the group consisting of Ser, Gly, Gln, Asp, Asn, Glu, Met, Ile, Pro, Arg, Thr, Lys, Val, Tyr and Phe.

CLMS(28)

28. The method of claim 3, wherein the scanning amino acid is selected from the group consisting of alanine, serine, glycine, and cysteine.

CLMS(29)

29. The method of claim 3, wherein the scanning amino acid is cysteine.

CLMS(30)

30. The method of claim 16, wherein the scanning amino acid is selected from the group consisting of Ser, Gly, Gln, Asp, Asn, Glu, Met, Ile, Pro, Arg, Thr, Lys, Val, Tyr and Phe.

CLMS(31)

31. The method of claim 16, wherein the scanning amino acid is cysteine.

US PAT NO: 5,580,723 [IMAGE AVAILABLE]

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ABSTRACT:

The invention provides methods for the systematic analysis of the structure and function of polypeptides by identifying active domains which influence the activity of the polypeptide with a target substance. Such active domains are determined by substituting selected amino acid segments of the polypeptide with an analogous polypeptide segment from an analog to the polypeptide. The analog has a different activity with the target substance as compared to the parent polypeptide. The activities of the segment-substituted polypeptides are compared to the same activity for the parent polypeptide for the target. A comparison of such activities provides an indication of the location of the active domain in the parent polypeptide. The invention also provides methods for identifying the active amino acid residues within the active domain of the parent polypeptide. The method comprises substituting a scanning amino acid for one of the amino acid residues within the active domain of

the parent polypeptide and assaying the residue-substituted polypeptide so formed with a target substance. The invention further provides polypeptide variants comprising segment-substituted and residue-substituted growth hormones, prolactins and placental lactogens.

TITLE: Method for identifying active domains and amino acid residues in polypeptides and hormone variants

CLAIMS:

CLMS(1)

What is claimed is:

1. A method for identifying at least a first unknown active domain in a region of known amino acid sequence of a naturally occurring parent polypeptide, which parent polypeptide has been cloned and has a preidentified biological activity, said active domain being capable of interacting with a first target when said parent polypeptide is in its native folded form, which interaction is responsible for said biological activity, said method comprising:

- (a) comparing amino acid sequence or polypeptide structure in the region of known amino acid sequence of the parent polypeptide with amino acid sequence or polypeptide structure in a region of known amino acid sequence of an analog polypeptide to the parent polypeptide, said parent polypeptide and said analog both interacting with said first target, resulting in said biological activity, but having different interactions with said first target, or said analog interacting with a different target with which said parent polypeptide also interacts;
- (b) substituting DNA encoding a first analogous polypeptide segment from the analog to said parent polypeptide into DNA encoding substantially the full length parent polypeptide and expressing a first segment-substituted polypeptide;
- (c) contacting said first segment-substituted polypeptide with said first target to determine the interaction, if any, between said first target and said segment-substituted polypeptide;
- (d) repeating steps b) and c) using a second analogous polypeptide segment from an analog to said parent polypeptide to form at least a second segment-substituted polypeptide containing said second analogous polypeptide segment, which is different from said first analogous polypeptide segment; and
- (e) comparing the difference, if any, between the activity relative to said first target of said parent polypeptide and said first and second segment-substituted polypeptides as an indication of the location of said first active domain in said parent polypeptide.

CLMS(2)

2. The method of claim 1 wherein said unknown active domain comprises at least two discontinuous amino acid segments in the primary amino acid sequence of said parent polypeptide.

CLMS(3)

3. The method of claim 1 wherein at least a first selected polypeptide segment of said parent polypeptide replaced by said first analogous polypeptide segment of said analog contains at least one amino acid residue located on the surface of the native-folded form of said parent polypeptide.

CLMS(4)

4. The method of claim 3 further comprising repeating steps b) and c) until substantially all of the amino acid residues on said surface of said parent polypeptide have been substituted by said analogous polypeptide segments.

CLMS(5)

5. The method of claim 1 further comprising repeating steps b) and c) to form a plurality of segment-substituted polypeptides that, collectively, contain substitutions of analogous polypeptide segments covering about 15-100* of the amino acid sequence of said parent polypeptide.

CLMS(6)

6. The method of claim 1 further comprising repeating steps b) and c) to form a plurality of segment-substituted polypeptides that, collectively, contain substitutions of analogous-polypeptide segments covering about 60-100* of the amino acid sequence of said parent polypeptide.

CLMS(7)

7. The method of claim 1 further comprising identifying a second unknown active domain of said parent polypeptide, said second active domain interacting with a second target, said method comprising repeating steps b) through e) with said second target.

CLMS(8)

8. The method of claim 1 further comprising identifying at least a first active amino acid residue within said first active domain, said method comprising:

- f) substituting a scanning amino acid for a different first amino acid residue within said first active domain to form a first residue-substituted polypeptide;
- g) contacting said first residue-substituted polypeptide with said first target to determine the interaction, if any, between said target and said residue-substituted polypeptide;
- h) repeating steps f) and g) to substitute a scanning amino acid for at least a second amino acid residue within said first active domain to form at least a second residue-substituted polypeptide; and
- i) comparing the difference, if any, between the activity relative to said first target of the parent polypeptide and each of said first and second residue-substituted polypeptides as an indication of the location of said first active amino acid residue in said first active domain.

CLMS(9)

9. The method of claim 8 further comprising repeating steps (b) through (i) with a second target to identify a second active domain and at least one active amino acid residue within said second active domain.

CLMS(10)

10. The method of claim 9 further comprising the step of substituting at least one of said active amino acid residues in said first active domain with a different amino acid to produce a polypeptide variant having a modified interaction with said first target but which retains substantially all of the interaction of said parent polypeptide with said

second target.

CLMS(11)

11. The method of claim 10 further comprising the step of substituting at least one of said active amino acid residues in said second active domain with a different amino acid to produce a polypeptide variant having a modified interaction with said first and said second target.

CLMS(12)

12. The method of claim 9 wherein said first and said second active domains have at least one common active amino acid residue, said method further comprising substituting at least said one common active amino acid residue with a different amino acid to produce a polypeptide variant having modified interactions with each of said first and said second targets.

CLMS(13)

13. The method of claim 9 wherein said first and said second active domains have at least one common active amino acid residue, said method further comprising substituting at least one amino acid residue in said first active domain, other than said at least one common active amino acid residue, with a different amino acid to produce a polypeptide variant having a modified interaction with said first target.

CLMS(14)

14. The method of claim 1 further comprising:

- (f) substituting DNA encoding a scanning amino acid for DNA encoding a first amino acid residue at residue number N within DNA encoding substantially the full length parent polypeptide and expressing an N-substituted polypeptide;
- (g) substituting a scanning amino acid for each of the amino acid residues at residue numbers N+1 and N-1 to said first residue to form respectively N+1- and N-1-substituted polypeptides;
- (h) contacting each of said substituted polypeptides with said first target to determine the interaction, if any, between said first target and said substituted polypeptides;
- (i) comparing the difference, if any, between the activity relative to said first target of the parent polypeptide and said substituted polypeptides; and
- (j) repeating steps (g) through (i) for increasing residue numbers if said activity difference between said first target and said N+1-substituted polypeptide is greater than two-fold and for decreasing residue numbers if said activity difference between said first target and said N-1-substituted polypeptide is greater than two-fold.

CLMS(15)

15. The method of claim 14 wherein steps (g) through (i) are repeated until at least four substituted polypeptides containing the substitution of a scanning amino acid at four consecutive residues are identified having less than a two-fold activity difference as compared to said parent polypeptide.

CLMS(16)

16. The method of claim 1, 8 or 14 wherein said parent polypeptide is

selected from the group consisting of human growth hormone, human prolactin, .alpha.-interferon, .gamma.-interferon, tissue plasminogen activator, IGF-1, TGH-.beta..sub.1, EGF, CD-4, TNF, GMCSF, TGF, follicle stimulating hormone, luteinizing hormone, atrial natriuretic peptide and placental lactogen.

CLMS(17)

17. The method of claim 16 wherein said parent polypeptide is selected from the group consisting of human growth hormone, human placental lactogen and human prolactin.

CLMS(18)

18. The method of claim 8 or 14 wherein said scanning amino acid is an isosteric amino acid.

CLMS(19)

19. The method of claim 8 or 14 wherein said scanning amino acid is a neutral amino acid.

CLMS(20)

20. The method of claim 19 wherein said neutral amino acid is selected from the group consisting of alanine, serine, glycine and cysteine.

CLMS(21)

21. The method of claim 20 wherein said scanning amino acid is alanine.

CLMS(22)

22. The method of claim 1, 8 or 14 wherein said activity is measured in an in vitro or in vivo assay.

CLMS(23)

23. The method of claim 22 wherein said parent polypeptide is a hormone and said activity is measured in an in vitro assay using a soluble hormone receptor.

CLMS(24)

24. The method of claim 23 wherein said hormone is human growth hormone and said soluble hormone receptor is shGHR.

CLMS(25)

25. The method of claim 23 wherein said hormone is human growth hormone and said soluble hormone receptor is shPRLr.

CLMS(26)

26. The method of claims 1, 8 or 14 wherein said interaction between said first target and said parent polypeptide involves either binding of said target to said parent polypeptide or catalysis of said target by said parent polypeptide.

CLMS(27)

27. The method of claim 26 wherein the activity between said first target and any of said substituted polypeptides is increased greater than two-fold as compared to said parent polypeptide.

CLMS(28)

28. The method of claim 26 wherein the activity between said first target and any of said substituted polypeptides is decreased greater than two-fold as compared to said parent polypeptide.

CLMS(29)

29. The method of claim 1, wherein the analog has at least 15% amino acid sequence homology with the parent polypeptide.

CLMS(30)

30. The method of claim 1, wherein the analog is naturally occurring.

CLMS(31)

31. The method of claim 1, wherein the analog is a tertiary analog.

CLMS(32)

32. The method of claim 14 wherein said active domain comprises at least two discontinuous polypeptide segments in the primary amino acid sequence of said parent polypeptide.

CLMS(33)

33. The method of claim 1 wherein said parent polypeptide is human growth hormone and said analog is selected from the group consisting of human placental lactogen, porcine growth hormone, and human prolactin.

CLMS(34)

34. The method of claim 1 wherein the biological activity of the parent polypeptide is of clinical utility.

CLMS(35)

35. The method of claim 1 wherein the parent polypeptide is selected from the group consisting of a hormone, enzyme, antigen, receptor, enzyme substrate, binding protein, and enzyme inhibitor.

CLMS(36)

36. The method of claim 1 wherein said first target is selected from the group consisting of a hormone, enzyme, antibody, antigen, receptor, enzyme substrate, binding protein, and enzyme inhibitor.

=> s (chimeric enzyme? or variant enzyme?) and beta lactamase and analyte?

4525 CHIMERIC
68485 ENZYME?
23 CHIMERIC ENZYME?
(CHIMERIC(W) ENZYME?)
50495 VARIANT

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68485 ENZYME?
47 VARIANT ENZYME?
    (VARIANT(W) ENZYME?)
178933 BETA
3171 LACTAMASE
3011 BETA LACTAMASE
    (BETA(W) LACTAMASE)
6347 ANALYTE?
L3      0 (CHIMERIC ENZYME? OR VARIANT ENZYME?) AND BETA LACTAMASE AN
D A
    ANALYTE?

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=> d 12 12

12. 5,580,723, Dec. 3, 1996, Method for identifying active domains and amino acid residues in polypeptides and hormone variants; James A. Wells, et al., 435/6, 7.1, 69.1, 71.1; 436/501; 530/387.1, 388.1, 399, 806, 808 [IMAGE AVAILABLE]

=> d 12 12 ab

US PAT NO: 5,580,723 [IMAGE AVAILABLE] L2: 12 of 18

ABSTRACT:

The invention provides methods for the systematic analysis of the structure and function of polypeptides by identifying active domains which influence the activity of the polypeptide with a target substance. Such active domains are determined by substituting selected amino acid segments of the polypeptide with an analogous polypeptide segment from an analog to the polypeptide. The analog has a different activity with the target substance as compared to the parent polypeptide. The activities of the segment-substituted polypeptides are compared to the same activity for the parent polypeptide for the target. A comparison of such activities provides an indication of the location of the active domain in the parent polypeptide. The invention also provides methods for identifying the active amino acid residues within the active domain of the parent polypeptide. The method comprises substituting a scanning amino acid for one of the amino acid residues within the active domain of the parent polypeptide and assaying the residue-substituted polypeptide so formed with a target substance. The invention further provides polypeptide variants comprising segment-substituted and residue-substituted growth hormones, prolactins and placental lactogens.

=> s target and binding and beta lactamase

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116734 TARGET
107967 BINDING
178933 BETA
3171 LACTAMASE
3011 BETA LACTAMASE
    (BETA(W) LACTAMASE)
L4      1000 TARGET AND BINDING AND BETA LACTAMASE

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=> s target and binding and beta lactamase and antibod?

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116734 TARGET
107967 BINDING
178933 BETA

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3171 LACTAMASE
3011 BETA LACTAMASE
      (BETA(W)LACTAMASE)
33432 ANTIBOD?
L5      883 TARGET AND BINDING AND BETA LACTAMASE AND ANTIBOD?

=> s target and binding and beta lactamase and antibod? and analyt?

116734 TARGET
107967 BINDING
178933 BETA
      3171 LACTAMASE
      3011 BETA LACTAMASE
            (BETA(W)LACTAMASE)
33432 ANTIBOD?
63517 ANALYT?
L6      330 TARGET AND BINDING AND BETA LACTAMASE AND ANTIBOD? AND ANAL
YT?

=> d 16 50-100

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50. 5,834,250, Nov. 10, 1998, Method for identifying active domains and amino acid residues in polypeptides and hormone variants; James A. Wells, et al., 435/7.1, 4, 6; 436/501; 530/300, 326 [IMAGE AVAILABLE]

51. 5,834,229, Nov. 10, 1998, Nucleic acids vectors and host cells encoding and expressing heregulin 2-.alpha.; Richard L. Vandlen, et al., 435/69.1, 91.2, 252.3, 320.1, 325; 536/23.5, 24.31, 24.33 [IMAGE AVAILABLE]

52. 5,834,183, Nov. 10, 1998, Gene sequence for spinocerebellar ataxia type 1 and method for diagnosis; Harry T. Orr, et al., 435/6, 91.2, 252.3, 320.1, 325; 536/23.1, 23.5, 24.31, 24.33 [IMAGE AVAILABLE]

53. 5,830,858, Nov. 3, 1998, Neurotrophic factor; Arnon Rosenthal, 514/12; 435/69.1; 514/2; 530/350, 395, 399, 402 [IMAGE AVAILABLE]

54. 5,830,713, Nov. 3, 1998, Methods for preparing synthetic repetitive DNA; Franco A. Ferrari, et al., 435/91.1, 6, 69.1, 252.8, 320.1; 536/23.1, 24.1, 25.3 [IMAGE AVAILABLE]

55. 5,830,647, Nov. 3, 1998, Hybridization and amplification of nucleic acids encoding mpl ligand; Dan L. Eaton, et al., 435/6, 91.1, 91.2, 91.5; 530/351, 399; 536/24.3, 24.31, 24.32, 24.33 [IMAGE AVAILABLE]

56. 5,827,734, Oct. 27, 1998, Materials and methods for determining ob protein in a biological sample; David S. Weigle, et al., 435/325, 7.1, 7.92, 7.94; 530/387.1 [IMAGE AVAILABLE]

57. 5,824,508, Oct. 20, 1998, Non-splicing variants of gp350/220; Richard Spaete, et al., 435/69.3; 424/230.1; 435/252.3, 254.21, 320.1, 325; 536/23.72 [IMAGE AVAILABLE]

58. 5,821,337, Oct. 13, 1998, Immunoglobulin variants; Paul J. Carter, et al., 530/387.3; 424/133.1; 530/350, 388.2 [IMAGE AVAILABLE]

59. 5,821,333, Oct. 13, 1998, Method for making heteromultimeric polypeptides; Paul J. Carter, et al., 530/350; 435/69.1, 69.7, 70.1, 71.1; 530/300, 387.1, 387.3 [IMAGE AVAILABLE]

60. 5,817,482, Oct. 6, 1998, Disease related nucleotide kinases; Olga Bandman, et al., 435/69.1, 6, 194, 252.3, 320.1; 530/350; 536/23.1, 23.5, 24.31, 24.33 [IMAGE AVAILABLE]
61. 5,811,524, Sep. 22, 1998, Neutralizing high affinity human monoclonal ****antibodies**** specific to RSV F-protein and methods for their manufacture and therapeutic use thereof; Peter Brams, et al., 530/388.3; 424/133.1, 147.1; 536/23.53 [IMAGE AVAILABLE]
62. 5,811,393, Sep. 22, 1998, Heparin ****binding**** mitogen with homology to epidermal growth factor (EGF); Michael Klagsbrun, et al., 514/12; 435/69.1, 320.1, 325; 530/324, 350, 399; 536/23.5 [IMAGE AVAILABLE]
63. 5,811,284, Sep. 22, 1998, Nucleic acids encoding kp43 protein and antigenic fragments thereof; Chiwen Chang, et al., 435/252.3, 69.1, 320.1, 325; 536/23.1, 23.5, 24.3, 24.31, 24.33 [IMAGE AVAILABLE]
64. 5,811,245, Sep. 22, 1998, ****Antibodies**** that specifically bind to ALK-7, a novel serine threonine kinase receptor; Carlos F. Ibanez, et al., 435/7.2, 334, 346, 975; 530/387.1, 387.9, 388.1 [IMAGE AVAILABLE]
65. 5,811,104, Sep. 22, 1998, Recombinant structural and non-structural proteins of FIPV and method of immunizing; Beverly Dale, et al., 424/221.1, 186.1, 199.1, 204.1; 435/69.3, 320.1 [IMAGE AVAILABLE]
66. 5,808,077, Sep. 15, 1998, Intercalators having affinity for DNA and methods of use; Christopher Bieniarz, et al., 546/165 [IMAGE AVAILABLE]
67. 5,808,001, Sep. 15, 1998, Human ice homolog ****antibodies**** and compositions thereof; Scott Michael Braxton, et al., 530/387.1, 388.1 [IMAGE AVAILABLE]
68. 5,807,706, Sep. 15, 1998, Method for making heteromultimeric polypeptides; Paul J. Carter, et al., 435/69.1, 69.7, 70.1, 71.1; 530/300, 350, 387.1, 387.3 [IMAGE AVAILABLE]
69. 5,804,425, Sep. 8, 1998, Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases; Gerard Francis Barry, et al., 435/193, 252.3, 252.33, 320.1, 822, 839, 874, 883 [IMAGE AVAILABLE]
70. 5,804,376, Sep. 8, 1998, Pancreas-derived serpin; Scott Michael Braxton, et al., 435/6, 69.1, 91.2, 348, 358, 369; 536/23.1, 24.3, 24.33, 24.5 [IMAGE AVAILABLE]
71. 5,801,236, Sep. 1, 1998, Probes for MTS1 gene and polynucleotides encoding mutant MTS1 genes; Alexander Kamb, 536/24.31; 435/320.1; 536/23.1, 24.3, 24.33 [IMAGE AVAILABLE]
72. 5,798,448, Aug. 25, 1998, AL-1 neurotrophic factor ****antibodies****; Ingrid W. Caras, et al., 530/387.1; 424/9.34, 130.1, 132.1, 133.1, 134.1, 135.1, 136.1; 435/7.1, 7.2, 7.9; 436/512, 514, 517, 518, 536, 538, 547, 548; 530/300, 350, 387.3, 387.9, 388.1, 388.15, 388.24, 389.1 [IMAGE AVAILABLE]
73. 5,798,246, Aug. 25, 1998, Cyclic nucleotide phosphodiesterase; Janice Au-Young, et al., 435/196, 320.1, 325; 536/23.2, 23.5 [IMAGE AVAILABLE]
74. 5,798,233, Aug. 25, 1998, Glycosyltransferases for biosynthesis of

- oligosaccharides, and genes encoding them; Emil C. Gotschlich, 435/97, 193 [IMAGE AVAILABLE]
75. 5,792,850, Aug. 11, 1998, Hematopoietic cytokine receptor; James W. Baumgartner, et al., 536/23.5; 435/69.5, 335 [IMAGE AVAILABLE]
76. 5,792,849, Aug. 11, 1998, Glial mitogenic factors, their preparation and use; Andrew Goodearl, et al., 536/23.5 [IMAGE AVAILABLE]
77. 5,792,617, Aug. 11, 1998, Cell proliferation-based amplified detection of **analytes**; M. Boris Rotman, 435/7.23, 5, 6, 7.1, 7.2, 7.5, 7.9, 29, 34, 834, 839, 848, 878, 881, 883, 966; 436/527, 531, 807, 808, 813 [IMAGE AVAILABLE]
78. 5,792,456, Aug. 11, 1998, Mutant BR96 **antibodies** reactive with human carcinomas; Dale Yelton, et al., 424/133.1, 130.1, 141.1; 530/387.1, 387.3, 388.1, 388.8, 391.3, 391.7 [IMAGE AVAILABLE]
79. 5,789,565, Aug. 4, 1998, Serine threonine kinase receptor, ALK-7; Carlos F. Ibanez, et al., 536/23.5; 435/320.1, 325 [IMAGE AVAILABLE]
80. 5,789,198, Aug. 4, 1998, Human leptin receptor-related protein; Ingrid E. Akerblom, 435/69.1, 320.1, 325; 536/23.5 [IMAGE AVAILABLE]
81. 5,789,192, Aug. 4, 1998, Mammalian receptors for interleukin-10 (IL-10); Kevin W. Moore, et al., 435/69.1, 252.3, 320.1; 536/23.5, 24.3 [IMAGE AVAILABLE]
82. 5,786,210, Jul. 28, 1998, Mammalian thymokine genes; Gregory S. Kelner, et al., 435/320.1, 252.3, 325; 536/23.5 [IMAGE AVAILABLE]
83. 5,783,669, Jul. 21, 1998, Hyaluronan receptor expressed in human umbilical vein endothelial; Phillip R. Hawkins, et al., 530/350; 435/69.1; 536/23.5 [IMAGE AVAILABLE]
84. 5,783,415, Jul. 21, 1998, Method of producing an IL-8 receptor polypeptide; James Lee, et al., 435/69.1, 71.1, 252.3, 320.1, 325; 530/350 [IMAGE AVAILABLE]
85. 5,780,426, Jul. 14, 1998, Fivemer cyclic peptide inhibitors of diseases involving .alpha..sub.v .beta..sub.3; Michael A. Palladino, et al., 514/9, 11, 17; 530/317, 329 [IMAGE AVAILABLE]
86. 5,780,268, Jul. 14, 1998, Chemokine expressed in a mixed lymphocyte reaction; Roger Coleman, et al., 435/69.5, 320.1, 325; 536/23.5 [IMAGE AVAILABLE]
87. 5,780,266, Jul. 14, 1998, Feline infectious peritonitis virus diagnostic tools; Beverly Dale, et al., 435/69.3, 252.3, 320.1, 325; 536/23.72 [IMAGE AVAILABLE]
88. 5,780,025, Jul. 14, 1998, Compositions and methods for the synthesis and assay of enkephalinase; Bernard Malfroy-Camine, et al., 424/94.67; 435/212 [IMAGE AVAILABLE]
89. 5,776,457, Jul. 7, 1998, **Antibodies** to human PF4A receptor and compositions thereof; James Lee, et al., 424/139.1, 143.1; 435/7.24; 530/387.9, 388.22, 389.6 [IMAGE AVAILABLE]
90. 5,773,580, Jun. 30, 1998, Human protein kinase c inhibitor homolog;

Janice Au-Young, et al., 530/350 [IMAGE AVAILABLE]

91. 5,773,435, Jun. 30, 1998, Prodrugs for **beta**-**lactamase** and uses thereof; John Kadow, et al., 514/214; 540/222, 225, 226 [IMAGE AVAILABLE]

92. 5,773,223, Jun. 30, 1998, Endothelin B.sub.1, (ETB.sub.1) receptor polypeptide and its encoding nucleic acid methods, and uses thereof; Venkatakrishna Shyamala, et al., 435/7.2, 69.1; 530/350; 536/23.5 [IMAGE AVAILABLE]

93. 5,770,704, Jun. 23, 1998, Receptor activation with inactive hepatocyte growth factor ligands; Paul J. Godowski, 530/402; 424/194.1, 195.11; 530/399 [IMAGE AVAILABLE]

94. 5,770,697, Jun. 23, 1998, Peptides comprising repetitive units of amino acids and DNA sequences encoding the same; Franco A. Ferrari, et al., 530/353; 435/69.1; 530/356, 357 [IMAGE AVAILABLE]

95. 5,770,567, Jun. 23, 1998, Sensory and motor neuron derived factor (SMDF); Wei-Hsien Ho, et al., 514/12, 2; 530/350, 395, 399 [IMAGE AVAILABLE]

96. 5,770,387, Jun. 23, 1998, **Antibodies** to mammalian NK antigens and uses; Virginia M. Litwin, et al., 435/7.24, 975; 530/388.3, 388.73, 389.6, 391.1, 391.3, 391.5 [IMAGE AVAILABLE]

97. 5,767,100, Jun. 16, 1998, Compounds and methods for making and using same; Petr Alexander, et al., 514/44; 435/6, 91.2; 514/261; 536/22.1, 25.3, 26.1; 544/313; 549/417 [IMAGE AVAILABLE]

98. 5,767,071, Jun. 16, 1998, Sevenmer cyclic peptide inhibitors of diseases involving **alpha**.sub.v **beta**.sub.3; Michael A. Palladino, et al., 514/11, 15; 530/317, 328 [IMAGE AVAILABLE]

99. 5,767,063, Jun. 16, 1998, Human IL-8 receptor and **antibodies** thereto; James Lee, et al., 514/2; 435/69.1, 69.7; 514/8, 12; 530/350, 387.1, 387.9, 388.22 [IMAGE AVAILABLE]

100. 5,763,584, Jun. 9, 1998, Receptor activation with hepatocyte growth factor agonists; Paul J. Godowski, 530/402; 424/194.1, 195.11; 530/399 [IMAGE AVAILABLE]

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L3          0 S (CHIMERIC ENZYME? OR VARIANT ENZYME?) AND BETA LACTAMASE
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L4          1000 S TARGET AND BINDING AND BETA LACTAMASE
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L8          291 L7 AND TARGET?

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1. 5,866,344, Feb. 2, 1999, ****Antibody**** selection methods using cell surface expressed libraries; George Georgiou, 435/7.21, 7.1, 7.32 [IMAGE AVAILABLE]

2. 5,866,341, Feb. 2, 1999, Compositions and methods for screening drug libraries; Dominic Gregory Spinella, et al., 435/7.1, 4, 5, 6, 7.2, 7.92 [IMAGE AVAILABLE]

3. 5,866,332, Feb. 2, 1999, Human myeloid terminal differentiation response gene; Benjamin Graeme Cocks, et al., 435/6, 69.1, 91.2, 252.3, 320.1, 325; 514/44; 536/23.5 [IMAGE AVAILABLE]

4. 5,866,125, Feb. 2, 1999, Neutralizing high affinity human monoclonal ****antibodies**** specific to RSV F-protein and methods for their manufacture and therapeutic use thereof; Peter Brams, et al., 424/133.1, 147.1; 536/23.53 [IMAGE AVAILABLE]

5. 5,864,020, Jan. 26, 1999, HTK ligand; Brian D. Bennett, et al., 530/388.24; 435/188; 530/387.1, 391.1, 391.3 [IMAGE AVAILABLE]

6. 5,863,796, Jan. 26, 1999, ****Antibodies**** which specifically bind mammalian receptors for interleukin-10 (IL-10); Kevin W. Moore, et al., 435/331; 424/139.1, 141.1, 143.1; 435/7.21, 334; 530/387.9, 388.2, 388.22, 388.85, 389.1, 389.6, 391.1, 391.3 [IMAGE AVAILABLE]

7. 5,863,779, Jan. 26, 1999, UBC7-like ubiquitin-conjugating enzyme; Preeti Lal, et al., 435/193, 252.3, 320.1; 536/23.2 [IMAGE AVAILABLE]

8. 5,859,206, Jan. 12, 1999, ****Antibodies**** specific for heregulin 2- α .; Richard L. Vandlen, et al., 530/387.9; 424/139.1, 141.1; 530/387.3, 388.2, 388.24, 388.85, 389.1, 389.2, 391.1, 391.3 [IMAGE AVAILABLE]

9. 5,858,967, Jan. 12, 1999, Appetite supression factor and related

methods; David S. Weigle, et al., 514/2; 435/69.1 [IMAGE AVAILABLE]

10. 5,858,751, Jan. 12, 1999, Compositions and methods for producing sialyltransferases; James C. Paulson, et al., 435/193, 69.1, 252.3, 320.1; 530/350; 536/23.2 [IMAGE AVAILABLE]

11. 5,858,708, Jan. 12, 1999, Polynucleotides encoding two novel human neuroendocrine-specific proteins; Olga Bandman, et al., 435/69.1, 320.1, 325; 536/23.1, 23.5 [IMAGE AVAILABLE]

12. 5,858,707, Jan. 12, 1999, Purified mammalian nucleobase permeases; nucleic acids; **antibodies**; M. Jorge Guimaraes, et al., 435/69.1, 91.1, 320.1; 536/23.5 [IMAGE AVAILABLE]

13. 5,856,457, Jan. 5, 1999, Nucleic acids encoding a human IL-8 receptor; James Lee, et al., 536/23.5; 435/70.1, 252.3, 325; 530/350 [IMAGE AVAILABLE]

14. 5,856,437, Jan. 5, 1999, Hepatitis C virus isolate polypeptides; Tatsuo Miyamura, et al., 530/350; 435/5, 235.1; 530/300, 324, 325, 326, 327, 328, 329, 330, 403, 826 [IMAGE AVAILABLE]

15. 5,856,297, Jan. 5, 1999, Human C3b/C4b receptor (CR1); Douglas T. Fearon, et al., 514/2; 435/69.1; 530/350; 536/23.4 [IMAGE AVAILABLE]

16. 5,856,158, Jan. 5, 1999, Purified nitric oxide synthase from rat brain; John P. N. Rosazza, et al., 435/191; 424/570; 435/335 [IMAGE AVAILABLE]

17. 5,856,110, Jan. 5, 1999, Method of using HRG2-.alpha. to stimulate P185.sup.HeR2; Richard L. Vandlen, et al., 435/7.21; 514/12 [IMAGE AVAILABLE]

18. 5,856,109, Jan. 5, 1999, Homolog of rat elastase IV derived from human pancreas; Scott M. Braxton, et al., 435/7.6; 530/350 [IMAGE AVAILABLE]

19. 5,856,090, Jan. 5, 1999, DNA-methylase linking reaction; David M. Epstein, 435/6, 7.1, 69.7, 320.1; 536/23.1 [IMAGE AVAILABLE]

20. 5,854,220, Dec. 29, 1998, Glial mitogenic factors, their preparation and use; Andrew Goodearl, et al., 514/12, 2; 530/350, 399 [IMAGE AVAILABLE]

21. 5,853,997, Dec. 29, 1998, Human protein phosphatase; Olga Bandman, et al., 435/6, 196, 252.3, 254.11, 320.1, 325, 410; 536/23.2 [IMAGE AVAILABLE]

22. 5,851,984, Dec. 22, 1998, Method of enhancing proliferation or differentiation of hematopoietic stem cells using Wnt polypeptides; William Matthews, et al., 514/2; 424/85.1; 435/2 [IMAGE AVAILABLE]

23. 5,849,533, Dec. 15, 1998, Method for making unclipped HIV envelope protein; Phillip W. Berman, et al., 435/69.3; 424/188.1, 208.1; 530/412, 413 [IMAGE AVAILABLE]

24. 5,849,498, Dec. 15, 1998, Human 3-hydroxyisobutyryl-coenzyme A hydrolase; Olga Bandman, et al., 435/6, 196, 252.3, 320.1; 536/23.2 [IMAGE AVAILABLE]

25. 5,846,818, Dec. 8, 1998, Pectate lyase signal sequence; Randy R. Robinson, et al., 435/320.1, 69.1, 69.7, 69.8, 252.3, 252.33; 536/23.4, 23.7 [IMAGE AVAILABLE]
26. 5,846,778, Dec. 8, 1998, Human map kinase homolog; Phillip R. Hawkins, et al., 435/69.1, 320.1; 530/350, 827; 536/23.5 [IMAGE AVAILABLE]
27. 5,846,777, Dec. 8, 1998, Two cDNAs encoding WD-40 proteins; Olga Bandman, et al., 435/69.1, 252.33, 320.1, 325; 536/23.1, 23.5 [IMAGE AVAILABLE]
28. 5,846,763, Dec. 8, 1998, DNA encoding tumor necrosis factor stimulated gene 6 (TSG-6); Tae Ho Lee, et al., 435/69.1, 252.3, 320.1; 536/23.1, 23.5 [IMAGE AVAILABLE]
29. 5,844,092, Dec. 1, 1998, Human TRK receptors and neurotrophic factor inhibitors; Leonard G. Presta, et al., 530/387.3; 424/133.1; 530/350 [IMAGE AVAILABLE]
30. 5,844,084, Dec. 1, 1998, Chemokine expressed in inflamed adenoid; Karl J. Guegler, et al., 530/351; 435/69.5, 71.2, 252.3, 320.1, 325; 536/23.5; 930/140 [IMAGE AVAILABLE]
31. 5,843,756, Dec. 1, 1998, Mouse MTSI gene; Steven Stone, et al., 435/252.3, 320.1, 325; 536/23.5, 24.31, 24.33 [IMAGE AVAILABLE]
32. 5,843,719, Dec. 1, 1998, Cellubrevin homolog; Susan G. Stuart, et al., 435/69.1; 530/350; 536/23.5 [IMAGE AVAILABLE]
33. 5,843,697, Dec. 1, 1998, Cells expressing IL-10 receptor and the CRFB4 gene product, an IL-10 receptor accessory protein; Sidney Pestka, et al., 435/29, 7.21, 320.1, 325; 536/23.5 [IMAGE AVAILABLE]
34. 5,840,856, Nov. 24, 1998, **Antibodies** to a human PF4 superfamily receptor; Anan Chuntharapai, et al., 530/388.22; 435/70.21; 530/387.9, 388.1 [IMAGE AVAILABLE]
35. 5,840,844, Nov. 24, 1998, Soluble lymphocyte homing receptors; Laurence A. Lasky, et al., 530/350; 424/158.1; 435/69.1 [IMAGE AVAILABLE]
36. 5,840,569, Nov. 24, 1998, Human GTP-**binding** proteins; Jennifer L. Hillman, et al., 435/252.3, 69.1, 320.1; 536/23.1, 23.5, 24.31, 24.32 [IMAGE AVAILABLE]
37. 5,840,562, Nov. 24, 1998, DNA encoding human cysteine protease; Dinh Diep, et al., 435/212, 252.33, 254.21, 320.1, 325; 536/23.1, 23.2, 23.5 [IMAGE AVAILABLE]
38. 5,840,535, Nov. 24, 1998, DNA encoding a zinc ring protein; Jennifer L. Hillman, et al., 435/69.1; 536/23.5, 24.31 [IMAGE AVAILABLE]
39. 5,840,525, Nov. 24, 1998, Nucleic acids, vectors and host cells encoding heregulin; Richard L. Vandlen, et al., 435/69.1, 252.3, 320.1, 325; 536/23.5 [IMAGE AVAILABLE]
40. 5,840,298, Nov. 24, 1998, Neutralizing high affinity human monoclonal **antibodies** specific to RSV F-protein and methods for their manufacture and therapeutic use thereof; Peter Brams, et al., 424/133.1, 147.1; 536/23.53 [IMAGE AVAILABLE]

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ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The recent development of catalytic **antibodies** and the introduction of new techniques to generate huge libraries Of random **mutants** Of existing enzymes have created the need for powerful tools for finding in large populations of cells those producing the catalytically most active proteins. Several approaches have been developed and used to reach this goal. The screening techniques aim at easily detecting the clones producing active enzymes or abzymes; the selection techniques are designed to extract these clones from mixtures: These techniques have been applied both in vivo and in vitro. This review describes the advantages and limitations Of the various methods in terms of ease of use, sensitivity, and convenience for handling large libraries.

Examples are analyzed and tentative rules proposed. These techniques prove to be quite powerful to study the relationship between structure and function and to alter the properties of enzymes.

TITLE: Activation of prodrugs by **enzymes** that are fusion products of catalytic domains and antigen-
binding domains

INVENTOR(S): Gehrmann, Mathias; Seemann, Gerhard; Bosslet, Klaus; Czech, Joerg

PATENT ASSIGNEE(S): Behringwerke Aktiengesellschaft, Germany

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AU 9348791	A1	19940414	AU 93-48791	19931004
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AB Activation of prodrugs of cytotoxins at a defined target site is achieved using a fusion protein of prodrug-activating **enzyme** and an antigen-**binding** domain specific for an antigen of the target site. A fusion protein of a humanized sFv fragment of an **antibody** to carcinoembryonic antigen and a human .beta.-glucuronidase was prepd.

by expression of the **chimeric** gene in BHK cells. The protein was shown to specifically bind CEA and to hydrolyze 4-methyl-umbelliferyl-.beta.-glucuronide. Manuf. of the fusion protein in yeast is demonstrated. Pharmacokinetics of the fusion protein 0.8 .mu.g injected into tumor-bearing nude mice showed very rapid clearing of the protein from the plasma and all other organs. The highest levels of the protein were found in the tumor with a concn. of 6.2 ng/g tissue after 120 h compared to <0.1 ng/g for all other tissue tested.

55. 5,824,508, Oct. 20, 1998, Non-splicing ****variants**** of gp350/220; Richard Spaete, et al., 435/69.3; 424/230.1; 435/252.3, 254.21, 320.1, 325; 536/23.72 [IMAGE AVAILABLE]
56. 5,821,337, Oct. 13, 1998, Immunoglobulin ****variants****; Paul J. Carter, et al., 530/387.3; 424/133.1; 530/350, 388.2 [IMAGE AVAILABLE]
57. 5,821,333, Oct. 13, 1998, Method for making heteromultimeric polypeptides; Paul J. Carter, et al., 530/350; 435/69.1, 69.7, 70.1, 71.1; 530/300, 387.1, 387.3 [IMAGE AVAILABLE]
58. 5,817,482, Oct. 6, 1998, Disease related nucleotide kinases; Olga Bandman, et al., 435/69.1, 6, 194, 252.3, 320.1; 530/350; 536/23.1, 23.5, 24.31, 24.33 [IMAGE AVAILABLE]
59. 5,811,524, Sep. 22, 1998, Neutralizing high affinity human monoclonal ****antibodies**** specific to RSV F-protein and methods for their manufacture and therapeutic use thereof; Peter Brams, et al., 530/388.3; 424/133.1, 147.1; 536/23.53 [IMAGE AVAILABLE]
60. 5,811,284, Sep. 22, 1998, Nucleic acids encoding kp43 protein and antigenic fragments thereof; Chiwen Chang, et al., 435/252.3, 69.1, 320.1, 325; 536/23.1, 23.5, 24.3, 24.31, 24.33 [IMAGE AVAILABLE]
61. 5,811,245, Sep. 22, 1998, ****Antibodies**** that specifically bind to ALK-7, a novel serine threonine kinase receptor; Carlos F. Ibanez, et al., 435/7.2, 334, 346, 975; 530/387.1, 387.9, 388.1 [IMAGE AVAILABLE]
62. 5,811,104, Sep. 22, 1998, Recombinant structural and non-structural proteins of FIPV and method of immunizing; Beverly Dale, et al., 424/221.1, 186.1, 199.1, 204.1; 435/69.3, 320.1 [IMAGE AVAILABLE]
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65. 5,804,425, Sep. 8, 1998, Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases; Gerard Francis Barry, et al., 435/193, 252.3, 252.33, 320.1, 822, 839, 874, 883 [IMAGE AVAILABLE]
66. 5,804,376, Sep. 8, 1998, Pancreas-derived serpin; Scott Michael Braxton, et al., 435/6, 69.1, 91.2, 348, 358, 369; 536/23.1, 24.3, 24.33, 24.5 [IMAGE AVAILABLE]
67. 5,801,236, Sep. 1, 1998, Probes for MTS1 gene and polynucleotides encoding ****mutant**** MTS1 genes; Alexander Kamb, 536/24.31; 435/320.1; 536/23.1, 24.3, 24.33 [IMAGE AVAILABLE]
68. 5,798,448, Aug. 25, 1998, AL-1 neurotrophic factor ****antibodies****; Ingrid W. Caras, et al., 530/387.1; 424/9.34, 130.1, 132.1, 133.1, 134.1, 135.1, 136.1; 435/7.1, 7.2, 7.9; 436/512, 514, 517, 518, 536, 538, 547, 548; 530/300, 350, 387.3, 387.9, 388.1, 388.15, 388.24, 389.1 [IMAGE AVAILABLE]
69. 5,798,246, Aug. 25, 1998, Cyclic nucleotide phosphodiesterase; Janice Au-Young, et al., 435/196, 320.1, 325; 536/23.2, 23.5 [IMAGE AVAILABLE]

41. 5,837,815, Nov. 17, 1998, PYK2 related polypeptide products; Sima Lev, et al., 530/350; 435/69.1; 530/412 [IMAGE AVAILABLE]
42. 5,837,524, Nov. 17, 1998, PYK2 related polynucleotide products; Joseph Schlessinger, et al., 435/252.3, 91.4, 320.1; 536/23.1, 25.4 [IMAGE AVAILABLE]
43. 5,837,500, Nov. 17, 1998, Directed evolution of novel **binding** proteins; Robert Charles Ladner, et al., 435/69.7, 91.1, 91.2, 471; 530/350, 412; 536/23.4 [IMAGE AVAILABLE]
44. 5,837,464, Nov. 17, 1998, Compositions and methods for determining anti-viral drug susceptibility and resistance and anti-viral drug screening; Daniel Capon, et al., 435/6, 320.1, 369 [IMAGE AVAILABLE]
45. 5,837,458, Nov. 17, 1998, Methods and compositions for cellular and metabolic engineering; Jeremy Minshull, et al., 435/6 [IMAGE AVAILABLE]
46. 5,834,591, Nov. 10, 1998, Polypeptides and **antibodies** useful for the diagnosis and treatment of pathogenic neisseria and other microorganisms having type 4 pilin; Staffan Normark, et al., 530/350; 424/242.1, 249.1, 250.1; 435/7.1, 7.3, 69.1, 69.3, 71.1; 530/300, 324, 325, 326 [IMAGE AVAILABLE]
47. 5,834,307, Nov. 10, 1998, Polynucleotides encoding CMV neutralizing proteins; Richard R. Spaete, et al., 435/320.1, 5, 69.1, 69.3, 252.3, 325; 536/23.72 [IMAGE AVAILABLE]
48. 5,834,250, Nov. 10, 1998, Method for identifying active domains and amino acid residues in polypeptides and hormone **variants**; James A. Wells, et al., 435/7.1, 4, 6; 436/501; 530/300, 326 [IMAGE AVAILABLE]
49. 5,834,229, Nov. 10, 1998, Nucleic acids vectors and host cells encoding and expressing heregulin 2-.alpha.; Richard L. Vandlen, et al., 435/69.1, 91.2, 252.3, 320.1, 325; 536/23.5, 24.31, 24.33 [IMAGE AVAILABLE]
50. 5,834,183, Nov. 10, 1998, Gene sequence for spinocerebellar ataxia type 1 and method for diagnosis; Harry T. Orr, et al., 435/6, 91.2, 252.3, 320.1, 325; 536/23.1, 23.5, 24.31, 24.33 [IMAGE AVAILABLE]
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51. 5,830,858, Nov. 3, 1998, Neurotrophic factor; Arnon Rosenthal, 514/12; 435/69.1; 514/2; 530/350, 395, 399, 402 [IMAGE AVAILABLE]
52. 5,830,713, Nov. 3, 1998, Methods for preparing synthetic repetitive DNA; Franco A. Ferrari, et al., 435/91.1, 6, 69.1, 252.8, 320.1; 536/23.1, 24.1, 25.3 [IMAGE AVAILABLE]
53. 5,830,647, Nov. 3, 1998, Hybridization and amplification of nucleic acids encoding mpl ligand; Dan L. Eaton, et al., 435/6, 91.1, 91.2, 91.5; 530/351, 399; 536/24.3, 24.31, 24.32, 24.33 [IMAGE AVAILABLE]
54. 5,827,734, Oct. 27, 1998, Materials and methods for determining ob protein in a biological sample; David S. Weigle, et al., 435/325, 7.1, 7.92, 7.94; 530/387.1 [IMAGE AVAILABLE]

AVAILABLE]

70. 5,798,233, Aug. 25, 1998, Glycosyltransferases for biosynthesis of oligosaccharides, and genes encoding them; Emil C. Gotschlich, 435/97, 193 [IMAGE AVAILABLE]

71. 5,792,850, Aug. 11, 1998, Hematopoietic cytokine receptor; James W. Baumgartner, et al., 536/23.5; 435/69.5, 335 [IMAGE AVAILABLE]

72. 5,792,849, Aug. 11, 1998, Glial mitogenic factors, their preparation and use; Andrew Goodearl, et al., 536/23.5 [IMAGE AVAILABLE]

73. 5,792,617, Aug. 11, 1998, Cell proliferation-based amplified detection of **analytes**; M. Boris Rotman, 435/7.23, 5, 6, 7.1, 7.2, 7.5, 7.9, 29, 34, 834, 839, 848, 878, 881, 883, 966; 436/527, 531, 807, 808, 813 [IMAGE AVAILABLE]

74. 5,792,456, Aug. 11, 1998, **Mutant** BR96 **antibodies** reactive with human carcinomas; Dale Yelton, et al., 424/133.1, 130.1, 141.1; 530/387.1, 387.3, 388.1, 388.8, 391.3, 391.7 [IMAGE AVAILABLE]

75. 5,789,565, Aug. 4, 1998, Serine threonine kinase receptor, ALK-7; Carlos F. Ibanez, et al., 536/23.5; 435/320.1, 325 [IMAGE AVAILABLE]

76. 5,789,198, Aug. 4, 1998, Human leptin receptor-related protein; Ingrid E. Akerblom, 435/69.1, 320.1, 325; 536/23.5 [IMAGE AVAILABLE]

77. 5,789,192, Aug. 4, 1998, Mammalian receptors for interleukin-10 (IL-10); Kevin W. Moore, et al., 435/69.1, 252.3, 320.1; 536/23.5, 24.3 [IMAGE AVAILABLE]

78. 5,786,210, Jul. 28, 1998, Mammalian thymokine genes; Gregory S. Kelner, et al., 435/320.1, 252.3, 325; 536/23.5 [IMAGE AVAILABLE]

79. 5,783,669, Jul. 21, 1998, Hyaluronan receptor expressed in human umbilical vein endothelial; Phillip R. Hawkins, et al., 530/350; 435/69.1; 536/23.5 [IMAGE AVAILABLE]

80. 5,783,415, Jul. 21, 1998, Method of producing an IL-8 receptor polypeptide; James Lee, et al., 435/69.1, 71.1, 252.3, 320.1, 325; 530/350 [IMAGE AVAILABLE]

81. 5,780,268, Jul. 14, 1998, Chemokine expressed in a mixed lymphocyte reaction; Roger Coleman, et al., 435/69.5, 320.1, 325; 536/23.5 [IMAGE AVAILABLE]

82. 5,780,266, Jul. 14, 1998, Feline infectious peritonitis virus diagnostic tools; Beverly Dale, et al., 435/69.3, 252.3, 320.1, 325; 536/23.72 [IMAGE AVAILABLE]

83. 5,780,025, Jul. 14, 1998, Compositions and methods for the synthesis and assay of enkephalinase; Bernard Malfroy-Camine, et al., 424/94.67; 435/212 [IMAGE AVAILABLE]

84. 5,776,457, Jul. 7, 1998, **Antibodies** to human PF4A receptor and compositions thereof; James Lee, et al., 424/139.1, 143.1; 435/7.24; 530/387.9, 388.22, 389.6 [IMAGE AVAILABLE]

85. 5,773,580, Jun. 30, 1998, Human protein kinase c inhibitor homolog; Janice Au-Young, et al., 530/350 [IMAGE AVAILABLE]

86. 5,773,223, Jun. 30, 1998, Endothelin B.sub.1, (ETB.sub.1) receptor polypeptide and its encoding nucleic acid methods, and uses thereof; Venkatakrishna Shyamala, et al., 435/7.2, 69.1; 530/350; 536/23.5 [IMAGE AVAILABLE]
87. 5,770,704, Jun. 23, 1998, Receptor activation with inactive hepatocyte growth factor ligands; Paul J. Godowski, 530/402; 424/194.1, 195.11; 530/399 [IMAGE AVAILABLE]
88. 5,770,697, Jun. 23, 1998, Peptides comprising repetitive units of amino acids and DNA sequences encoding the same; Franco A. Ferrari, et al., 530/353; 435/69.1; 530/356, 357 [IMAGE AVAILABLE]
89. 5,770,567, Jun. 23, 1998, Sensory and motor neuron derived factor (SMDF); Wei-Hsien Ho, et al., 514/12, 2; 530/350, 395, 399 [IMAGE AVAILABLE]
90. 5,770,387, Jun. 23, 1998, ****Antibodies**** to mammalian NK antigens and uses; Virginia M. Litwin, et al., 435/7.24, 975; 530/388.3, 388.73, 389.6, 391.1, 391.3, 391.5 [IMAGE AVAILABLE]
91. 5,767,063, Jun. 16, 1998, Human IL-8 receptor and ****antibodies**** thereto; James Lee, et al., 514/2; 435/69.1, 69.7; 514/8, 12; 530/350, 387.1, 387.9, 388.22 [IMAGE AVAILABLE]
92. 5,763,584, Jun. 9, 1998, Receptor activation with hepatocyte growth factor agonists; Paul J. Godowski, 530/402; 424/194.1, 195.11; 530/399 [IMAGE AVAILABLE]
93. 5,763,213, Jun. 9, 1998, Sensory and motor neuron derived factor (SMDF); Wei-Hsien Ho, et al., 435/69.1, 320.1, 325; 536/23.5 [IMAGE AVAILABLE]
94. 5,759,816, Jun. 2, 1998, Expression vectors containing .lambda.P.sub.L promoter and T.sub.1 T.sub.2 rRNA termination sequence plasmids containing the vectors hosts containing the plasmids and related methods; Amos B. Oppenheim, et al., 435/69.4, 252.33, 320.1 [IMAGE AVAILABLE]
95. 5,759,775, Jun. 2, 1998, Methods for detecting nucleic acids encoding AL-1 neurotrophic factor; Ingrid W. Caras, et al., 435/6, 91.2; 536/23.5, 24.31, 24.33 [IMAGE AVAILABLE]
96. 5,756,456, May 26, 1998, Methods involving sensory and motor neuron derived factor (SMDF); Wei-Hsien Ho, et al., 514/12, 2 [IMAGE AVAILABLE]
97. 5,756,349, May 26, 1998, Production of erythropoietin; Fu-Kuen Lin, 435/325, 358, 365 [IMAGE AVAILABLE]
98. 5,753,516, May 19, 1998, Screening method for ligands of the EBI-1 receptor; Wyrtza E. Heagy, et al., 436/501; 435/6, 7.1, 7.2, 7.8 [IMAGE AVAILABLE]
99. 5,753,441, May 19, 1998, 170-linked breast and ovarian cancer susceptibility gene; Mark H. Skolnick, et al., 435/6; 424/1.11; 435/4, 7.1, 7.2, 7.9, 91.1, 91.2; 436/500, 548; 530/387.2, 388.1; 536/23.1, 24.3, 24.33 [IMAGE AVAILABLE]
100. 5,747,449, May 5, 1998, Bovine pancreatic trypsin inhibitor derived

- inhibitors of factor XA; Ignace Lasters, et al., 514/12; 530/324, 350 [IMAGE AVAILABLE]
101. 5,747,282, May 5, 1998, 17Q-linked breast and ovarian cancer susceptibility gene; Mark H. Skolnick, et al., 435/69.1, 6, 320.1, 325; 536/23.5, 24.31, 24.33 [IMAGE AVAILABLE]
102. 5,747,035, May 5, 1998, Polypeptides with increased half-life for use in treating disorders involving the LFA-1 receptor; Leonard G. Presta, et al., 424/144.1, 130.1, 133.1, 135.1, 141.1, 143.1, 153.1, 154.1, 173.1; 514/2, 8, 885; 530/387.1 [IMAGE AVAILABLE]
103. 5,744,584, Apr. 28, 1998, Antithrombosis agents; Robert M. Scarborough, 530/388.1; 435/7.21; 436/518, 536; 530/389.1 [IMAGE AVAILABLE]
104. 5,744,301, Apr. 28, 1998, Methods of detection of epstein barr virus induced genes expressed in the placenta; Mark Birkenbach, et al., 435/6, 5; 514/44; 536/23.5 [IMAGE AVAILABLE]
105. 5,741,667, Apr. 21, 1998, Tumor necrosis factor receptor-associated factors; David V. Goeddel, et al., 435/69.1, 252.3, 320.1; 536/23.5 [IMAGE AVAILABLE]
106. 5,739,277, Apr. 14, 1998, Altered polypeptides with increased half-life; Leonard G. Presta, et al., 530/326, 300, 350, 387.1 [IMAGE AVAILABLE]
107. 5,739,027, Apr. 14, 1998, MTS1E1.beta. gene; Alexander Kamb, 435/325, 252.33, 320.1, 358, 364, 367; 536/23.1, 24.31, 24.33 [IMAGE AVAILABLE]
108. 5,738,991, Apr. 14, 1998, Method of detecting elastase IV; Scott Michael Braxton, et al., 435/6, 320.1, 325; 536/23.1, 23.2, 23.5, 24.31 [IMAGE AVAILABLE]
109. 5,731,168, Mar. 24, 1998, Method for making heteromultimeric polypeptides; Paul J. Carter, et al., 435/69.1, 69.7, 70.1, 71.1; 530/300, 350, 387.1, 387.3; 536/23.1, 23.4, 23.5, 23.53 [IMAGE AVAILABLE]
110. 5,728,821, Mar. 17, 1998, **Mutant** BR96 **antibodies** reactive with human carcinomas; Dale Yelton, et al., 536/23.53; 424/141.1, 155.1; 435/70.21, 328, 344; 530/387.3, 388.8 [IMAGE AVAILABLE]
111. 5,726,290, Mar. 10, 1998, Soluble analogues of integrins; Sarah C. Bodary, et al., 530/350, 387.3 [IMAGE AVAILABLE]
112. 5,726,037, Mar. 10, 1998, Host cells and method of producing soluble analogues of integrins; Sarah C. Bodary, et al., 435/69.1, 69.7, 252.3, 325, 326 [IMAGE AVAILABLE]
113. 5,723,585, Mar. 3, 1998, Method of purifying cardiac hypertrophy factor; Joffre Baker, et al., 530/413, 350, 380; 930/140 [IMAGE AVAILABLE]
114. 5,717,058, Feb. 10, 1998, Peptide inhibitors of tax-dependent transcription; Maura-Ann H. Matthews, et al., 530/328, 324, 325, 326, 327, 330 [IMAGE AVAILABLE]
115. 5,716,930, Feb. 10, 1998, Glial growth factors; Andrew David

Goodearl, et al., 514/12; 435/69.1; 514/2; 530/350 [IMAGE AVAILABLE]

116. 5,716,810, Feb. 10, 1998, Nucleic acid encoding the mature .beta..sub.B chain of inhibin and method for synthesizing polypeptides using such nucleic acid; Anthony J. Mason, et al., 435/69.4, 69.1, 252.3, 325; 536/23.1, 23.5, 23.51 [IMAGE AVAILABLE]

117. 5,714,147, Feb. 3, 1998, Hybrid immunoglobulins; Daniel J. Capon, et al., 424/178.1; 435/69.7; 530/387.1; 536/23.7 [IMAGE AVAILABLE]

118. 5,710,001, Jan. 20, 1998, 17q-linked breast and ovarian cancer susceptibility gene; Mark H. Skolnick, et al., 435/6, 7.1, 7.9, 91.2; 530/300, 350, 388.1; 536/23.1, 24.3, 24.33 [IMAGE AVAILABLE]

119. 5,709,999, Jan. 20, 1998, Linked breast and ovarian cancer susceptibility gene; Donna M. Shattuck-Eidens, et al., 435/6, 91.2; 536/23.1, 24.3, 24.33 [IMAGE AVAILABLE]

120. 5,709,858, Jan. 20, 1998, **Antibodies** specific for Rse receptor protein tyrosine kinase; Paul J. Godowski, et al., 424/143.1, 139.1; 435/7.4; 530/387.3, 387.9, 388.22, 391.1, 391.3 [IMAGE AVAILABLE]

121. 5,708,149, Jan. 13, 1998, Method for producing purified recombinant Haemophilus influenzae transferrin **binding** proteins; Sheena Loosmore, et al., 530/418, 412, 413 [IMAGE AVAILABLE]

122. 5,708,142, Jan. 13, 1998, Tumor necrosis factor receptor-associated factors; David V. Goeddel, et al., 530/350; 435/69.1, 252.3, 320.1; 536/23.5 [IMAGE AVAILABLE]

123. 5,707,620, Jan. 13, 1998, Lysine-specific Porphyromonas gingivalis proteinase; James Travis, et al., 424/94.63; 435/7.1, 23, 220; 530/388.26, 389.4 [IMAGE AVAILABLE]

124. 5,705,367, Jan. 6, 1998, Glycosyltransferases for biosynthesis of oligosaccharides, and genes encoding them; Emil C. Gotschlich, 435/97, 193 [IMAGE AVAILABLE]

125. 5,703,213, Dec. 30, 1997, Monoclonal **antibodies** which recognize an adenocarcinoma cell antigen; Jack R. Wands, et al., 530/388.85; 435/70.21, 344.1; 530/387.7, 388.8 [IMAGE AVAILABLE]

126. 5,702,906, Dec. 30, 1997, **Antibodies** to neurotrophic factor-4 (NT-4); Arnon Rosenthal, 435/7.1, 236, 336; 530/387.1, 387.9, 388.24, 413 [IMAGE AVAILABLE]

127. 5,700,924, Dec. 23, 1997, Serpin derived from human hypothalamus; Scott Michael Braxton, et al., 536/23.1; 435/69.1, 91.4, 320.1 [IMAGE AVAILABLE]

128. 5,698,686, Dec. 16, 1997, Yeast telomerase compositions; Daniel E. Gottschling, et al., 536/23.1; 435/6, 91.2; 536/22.1, 24.3, 24.31, 24.33 [IMAGE AVAILABLE]

129. 5,698,435, Dec. 16, 1997, Modular assembly of **antibody** genes, **antibodies** prepared thereby and use; Randy R. Robinson, et al., 1/1; 435/69.1, 69.6, 235.1; 536/23.1, 23.53 [IMAGE AVAILABLE]

130. 5,698,417, Dec. 16, 1997, Modular assembly of **antibody** genes, **antibodies** prepared thereby and use; Randy R. Robinson, et al.,

435/69.1, 69.6; 536/23.1, 23.53 [IMAGE AVAILABLE]

131. 5,698,390, Dec. 16, 1997, Hepatitis C immunoassays; Michael Houghton, et al., 435/5; 436/518, 820 [IMAGE AVAILABLE]

132. 5,693,493, Dec. 2, 1997, Modular assembly of **antibody** genes, **antibodies** prepared thereby and use; Randy R. Robinson, et al., 435/69.1, 69.6 [IMAGE AVAILABLE]

133. 5,693,473, Dec. 2, 1997, Linked breast and ovarian cancer susceptibility gene; Donna M. Shattuck-Eidens, et al., 435/6, 91.2; 536/23.1, 24.3, 24.33 [IMAGE AVAILABLE]

134. 5,688,763, Nov. 18, 1997, Compositions and methods for the synthesis of growth hormone receptor and growth hormone **binding** protein; R. Glenn Hammonds, Jr., et al., 514/12, 2; 530/350 [IMAGE AVAILABLE]

135. 5,688,513, Nov. 18, 1997, Coccidiosis vaccines; Mary-Helen Binger, et al., 424/271.1, 184.1, 185.1, 265.1, 266.1; 530/350 [IMAGE AVAILABLE]

136. 5,686,597, Nov. 11, 1997, Thrombin receptor homolog; Roger Coleman, et al., 536/23.5; 435/2, 7.21, 69.1, 252.3, 254.11, 325, 348, 361, 365, 369 [IMAGE AVAILABLE]

137. 5,686,572, Nov. 11, 1997, Domains of extracellular region of human platelet derived growth factor receptor polypeptides; David Wolf, et al., 530/350; 435/69.1; 530/402 [IMAGE AVAILABLE]

138. 5,686,257, Nov. 11, 1997, **Binding** compositions for mammalian T cell antigens and related reagents; Jacqueline Kennedy, et al., 435/7.24, 7.5; 436/518; 530/387.9, 388.1, 388.75, 389.6, 391.1, 391.7, 402, 413 [IMAGE AVAILABLE]

139. 5,684,136, Nov. 4, 1997, Chimeric hepatocyte growth factor (HGF) ligand **variants**; Paul J. Godowski, 530/399, 387.3 [IMAGE AVAILABLE]

140. 5,681,819, Oct. 28, 1997, Method and compositions for reducing cholesterol absorption; Jordan J. N. Tang, et al., 514/12, 13, 14, 15, 16, 17, 18 [IMAGE AVAILABLE]

141. 5,679,545, Oct. 21, 1997, Gene encoding cardiac hypertrophy factor; Joffre Baker, et al., 435/69.1, 252.3, 320.1, 325; 536/23.5 [IMAGE AVAILABLE]

142. 5,679,542, Oct. 21, 1997, Antithrombosis agents; Robert M. Scarborough, 435/69.1, 252.3, 320.1, 325, 348, 419; 536/23.5 [IMAGE AVAILABLE]

143. 5,676,946, Oct. 14, 1997, Phospholipase C homolog; Phillip R. Hawkins, et al., 424/94.6; 435/69.1, 198, 252.33, 320.1; 536/23.2 [IMAGE AVAILABLE]

144. 5,674,984, Oct. 7, 1997, Method for isolation of unclipped HIV envelope protein; Phillip W. Berman, et al., 530/413, 388.35, 412 [IMAGE AVAILABLE]

145. 5,674,738, Oct. 7, 1997, DNA encoding thermostable nucleic acid polymerase enzyme from thermus species 205; Richard D. Abramson, et al., 435/252.3, 252.33, 320.1, 325, 419; 536/23.2 [IMAGE AVAILABLE]

146. 5,670,319, Sep. 23, 1997, Assay for tumor necrosis factor receptor-associated factors; David V. Goeddel, et al., 435/6, 7.1, 7.2, 69.7; 536/23.4 [IMAGE AVAILABLE]

147. 5,667,780, Sep. 16, 1997, **Antibodies** to SMDF; Wei-Hsien Ho, et al., 424/139.1; 530/387.3, 387.9, 388.23, 388.85, 389.2, 391.3 [IMAGE AVAILABLE]

148. 5,665,568, Sep. 9, 1997, Nucleic acid encoding the mature .beta..sub.A chain of inhibin and method for synthesizing polypeptides using such nucleic acid; Anthony J. Mason, et al., 435/69.4, 69.1, 252.3, 320.1, 325, 350, 352, 354, 360, 364, 365.1, 367, 370; 536/23.1, 23.5, 23.51 [IMAGE AVAILABLE]

149. 5,665,353, Sep. 9, 1997, Composition containing an analog of haemophilus Hin47 with reduced protease activity; Sheena M. Loosmore, et al., 424/94.63, 94.64; 435/220; 514/2, 12 [IMAGE AVAILABLE]

150. 5,663,313, Sep. 2, 1997, Human map kinase homolog; Phillip R. Hawkins, et al., 536/23.1; 435/6, 325; 536/23.5, 24.31 [IMAGE AVAILABLE]

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L3 0 S (CHIMERIC ENZYME? OR VARIANT ENZYME?) AND BETA LACTAMASE

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L4 1000 S TARGET AND BINDING AND BETA LACTAMASE

L5 883 S TARGET AND BINDING AND BETA LACTAMASE AND ANTIBOD?

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L9 291 S L7 AND TARGET?